Antimicrobial resistance in *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Escherichia coli* from the Faroese population, correlation with antimicrobial use and comparison with Iceland and Denmark

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Sýklalyfjaónæmi hjá *Streptococcus pneumoniae*, *Streptococcus pyogenes* og *Escherichia coli* í Færeyjum, tengsl við sýklalyfjanotkun og samanburður við Ísland og Danmörku

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Ágrip

Landfræðileg einangrun Færeyja gerir eyjaklasann að ákjósanlegum stað fyrir rannsóknir á beratíðni baktería, klónasamsetningu þeirra og bólusetningarannsóknir. Engu að síður eru slíkar rannsóknir ekki fyrir hendi og takmarkaður aðgangur að nauðsynlegum gögnum hefur takmarkað slíkar rannsóknir. Þau nýju gögn og greiningar sem birtast í þessari ritgerð gefa mikilvægar upplýsingar um sýklalyfjaónæmi hjá þremur meinvaldandi bakteríum í mönnum – streptókokkum af flokki A (GAS), *Escherichia coli* og pneumókokkum í Færeyjum.

Upplýsingar um sölu sýklalyfja fengust frá Lyfjafræðingi Færeyja, Lyfjastofnun Íslands, NOMESCO og DANMAP. Hálsstrokum og þvagsýnum var safnað af heilsugæslulæknum frá sjúklingum á árunum 2009, 2010 og 2012. Nefkoksstrokum var safnað frá heilbrigðum börnum á leikskólum í janúar til mars á árunum 2009, 2010 og 2011. Pneumókokkar úr ífarandi sýkingum í Færeyjum fengust frá Statens Seruminstitut, Danmörku. Öll sýni voru greind og pneumókokkastofnar hjúpgreindir í tveimur rannsóknastofum, einni í Færeyjum og einni á Íslandi. Næmispróf voru gerð í samræmi við CLSI og EUCAST aðferðir og staðla.

Á árunum 1999 til 2011 var sala sýklalyfja mest á Íslandi, síðan Færeyjum en minnst í Danmörku með 21,8, 17,7 og 16,3 staðlaða dagskammta (DDD) á 1000 íbúa á dag (DID). Það sem

bar mest á milli var mikil notkun tetrasýklín lyfja á Íslandi. Einnig var marktækur munur á sölu undirflokka penisillína og makrólíða milli þessara landa. Eryþrómýsín var einkum ávísað á 0-4 ára börn í Færeyjum.

Tetrasýklín ónæmi hjá GAS frá Færeyskum sjúklingum minnkaði mikið frá 2009 til 2010 (37% til 10%) og eryþrómýsín ónæmi minnkaði á Íslandi frá 2008 (44%) til 2009 (5%). Í Færeyjum fannst minnkað næmi hjá 54% *E. coli* stofna, þar sem ampisillín ónæmi var algengast (46%), síðan súlfamethoxazol (39%), trímetóprím (27%) og trímetóprím/súlfamethoxazol (27%). Það var marktæk fylgni á milli sölu sýklalyfja og sýklalyfjaónæmis. Ný nálgun okkar með notkun tölfræðilíkans greindi sýklalyf í tvo flokka, þau sem leiddu til mikillar aukningar á ónæmi hjá *E. coli* og þau sem höfðu aðeins miðlungs áhrif. Af þeim 607 börnum sem tekin voru sýni frá til greiningar á pneumókokkum, þá reyndust 50% bera bakteríuna árið 2009, 40% 2010 og 42% 2011.Sýklalyfjaónæmi var sjaldgæft hjá pneumókokkum, bæði í berum og sjúklingum með ífarandi sjúkdóm. Fimm stofnar frá berum reyndust vera með minnkað næmi fyrir penisllíni (PNSP, 1,8%) og einn frá ífarandi sýkingum (1,7%). Algengustu hjúpgerðirnar í berum voru 6B og 6A árið 2009, 3 og 6C árið 2010 og 11 og 6C árið 2011, og í ífarandi sýkingum 7F og 3.

Álykta má að tengslin á milli sýklalyfjaónæmis og sýklalyfjanotkunar réttlæti endurmat á stefnu við val á sýklalyfjum við þvagfærasýkingum. Beratíðni pneumókokka í börnum á leikskólum er lág og sýklalyfjaónæmi sjaldgæft. Algengi

pneumókokka með minnkað næmi fyrir penisillíni var lágt í samanburði við Ísland og Danmörku. Bólusetningaráætlunin frá 2008 virðist hafa fækkað PCV-7/13 hjúpgerðum í ífarandi sýkingum og auk þess eru vísbendingar um hjúpgerðarbreytingar hjá pneumókokkum í berum. Tölfræðilíkanið auðveldar okkur að gera raunhæfar spár um þróun ónæmis samfara aukinni sölu sýklalyfja og gæti gagnast öðrum þjóðum í eftirliti þeirra með sýklalyfjaónæmi. Með því að bera saman niðurstöður tengdar færeysku þjóðinni við niðurstöður nágrannalandanna Íslands og Danmörku, fæst i fyrsta sinn mat á stöðu Færeyja í samhengi við þá alheimsógn sem stafar af fjölónæmum bakteríum.

Abstract

The geographic remoteness of the Faroe Islands makes the archipelago an ideal location for research on bacterial carriage, their clonality, and vaccine studies. However, previous studies are lacking and the limited availability of necessary data has limited such investigations. The novel data collection and analysis presented in this thesis provides valuable knowledge on the antibacterial resistances in the three human bacterial pathogens - Group A Streptococcus (GAS), *Escherichia coli* and pneumococci in the Faroes.

Data on antibacterial sales were compiled from the National Pharmacist in the Faroe Islands, Icelandic Medicines Agency, NOMESCO and DANMAP. Clinical oropharynx and urine samples were collected from patients by general practitioners in the years 2009, 2010 and in 2012. Nasopharyngeal swabs were collected from healthy children attending day-care centres from January to March in 2009, 2010 and 2011. Invasive pneumococcal isolates from the Faroe Islands were obtained from Statens Serum Institut in Denmark. All samples were identified and pneumococcal isolates were serotyped in two laboratories, one in the Faroe Islands and one in Iceland. Susceptibility testing was done according to CLSI and EUCAST standards using conventional methods.

During the period 1999 to 2011, antibacterial sales were highest in Iceland, followed by the Faroe Islands and Denmark – with 21.8, 17.7 and 16.3 daily defined dose (DDD)/1000 inhabitants/day (DID), respectively. The most noteworthy difference was the higher sales of tetracycline in Iceland. The sales of the sub-groups, penicillins and macrolides differed significantly between the three countries. Erythromycin was mainly prescribed to children aged 0-4 years in the Faroe Island.

Among GAS isolates from Faroese patients, the resistance of tetracycline decreased markedly between 2009 and 2010 (37% to 10%) and erythromycin resistance dropped in Iceland from 2008 (44%) to 2009 (5%). In the Faroe Islands, non-susceptibility was found in 54% of *E. coli* isolates with non-susceptibility to ampicillin being the most common (46%), followed by sulfamethoxazole (39%), trimethoprim (27%), and trimethoprim/sulfamethoxazole (27%). A significant correlation was found between antibacterial sales and antibacterial resistance.

Our novel logistic modelling approach identified two categories of antibacterial agents – those that result in a marked increase in *E. coli* resistance and those with a moderate impact.

Of the 607 children screened for pneumococci, 50% were carriers in 2009, 40% in 2010 and 42% in 2011. Antibiotic resistance in pneumococci was rare both in carriers and invasive disease patients. Five penicillin non-susceptible pneumococci (PNSPs) were found in carriers (1.8%) and one was found among

the invasive isolates (1.7%). The most common serotypes in carriage (6B and 6A in 2009, 3 and 6C in 2010 and 11 and 6C in 2011) and among invasive pneumococcal diseases (IPD), 7F and 3.

In conclusion, the association between antibacterial resistance and antibacterial use justifies a re-evaluation of antibacterial policies regarding treatment of UTIs. The pneumococcal carriage prevalence in children attending day-care centres is low and antibacterial resistance in pneumococci is presently rare. The prevalence of PNSPs was low compared to Iceland and Denmark. The established vaccine program in 2008 appears to have reduced incidence of PCV-7/13 serotype among IPD, and there is, furthermore, an indication of a subsequent serotype shift in pneumococcal carriage.

Our logistic model facilitates representative predictions of the future developments in resistance with increased antibiotic sales and provides a valuable tool which can be used by resistance monitoring programs in other communities. Furthermore, by comparing results related to the Faroese population with the neighboring countries, Iceland and Denmark, we provide the first assessment of our community's status with regards to the global multi-resistance threat.

Keywords: Faroe Islands, Iceland, antibacterial sale, antibacterial resistance, pneumococcal serotypes, *E. coli, Streptococcus pyogenes.*

Til míni hjørtu og lívskærleika, Julian & Jason

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Contents

List of abbreviations

AMC	Amoxicillin/clavulanic acid
AOM	Acute otitis media
ATC	Anatomical therapeutically chemical classification system
CLSI	Clinical & Laboratory Standards Institute
DCC	Day care centre
DDD	Defined daily dosage
DID	DDD/1000 inhabitants/day
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ESBL	Extended spectrum ß-lactamases
GAS	Group A Streptococcus (*Streptococcus pyogenes)*
GP	General practitioner
MIC	Minimal inhibitory concentration
IPD	Invasive pneumococcal disease
OM	Otitis media
PCD	Primary carnitine deficiency
PCV	pneumococcal conjugated vaccine
PCV-7	PCV 7-valent: 4, 6B, 9V, 14, 18C, 19F and 23F
PCV-10	PCV 10-valent: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F
PCV-13	PCV 13-valent: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F
PHiDCV	Protein conjugated pneumococcal vaccine

PNSP	Penicillin non-susceptible pneumococci
NVT	Non-vaccine serotype
SXT	Trimethoprim-sulfamethoxazole
UTI	Urinary tract infection

List of figures

List of tables

List of original papers

This thesis is based on the following original papers, which will be referred to by the Roman numerals (I-IV).

I. Marita Debess Magnussen, Thorolfur Guðnason, Ulrich Stab Jensen, Niels Frimodt-Møller, Karl G. Kristinsson. Antibacterial use in the Faroe Islands, Iceland and Denmark 1999-2011. *Scand J of Inf Dis,* 2014, 502-507.

II. Marita Debess Magnussen, Shahin Gaini, Hannes Gislason, Karl G. Kristinsson. Antibacterial resistance in *Streptococcus pyogenes* (GAS) from healthy carriers and tonsillitis patients and association with antibacterial sale in the Faroe Islands. *APMIS,* 2016, 124(4), 327-32.

III. Marita Debess Magnussen, Hannes Gislason, Shahin Gaini, Karl G. Kristinsson. Antibacterial Susceptibilities of *Escherichia coli* from Community-Acquired Urinary Tract Infections in the Faroe Islands, Associations with Antibacterial Sales, and Comparison with Iceland and Denmark. *Microb Drug Resist,* 2018 Jan/Feb; 24(1): 40-47. Epub 2017 May 24.

IV. Marita Debess Magnussen, Helga Erlendsdóttir, Shahin Gaini, Thorolfur Gudnason and Karl G. Kristinsson. *Streptococcus pneumoniae* - Antimicrobial Resistance and Serotypes of Strains Carried by Children and Causing Invasive Disease in the Faroe Islands. *Microb Drug Resist,* Published online: 19 June 2018.

Declaration of contribution

Marita Debess Magnussen started her PhD studies in 2009, which were performed along with her fulltime job as a consultant microbiologist at the Food and Veterinary Agency, and as a lecturer at the Faculty of Sciences, University of Faroe Islands. She was lecturing Biology BSc students in microbiology and is currently lecturing on microbiology at the Faculty of Health Sciences. During the PhD studies, Marita started her own Food and Environmental laboratory in Tórshavn.

During the PhD study, Helga Erlendsdóttir contributed her expertise to the laboratory work and in all four manuscripts.

Paper I: This study described the antibacterial sales in the Faroe Islands, Iceland and Denmark. It was initiated by Marita and supervisor Karl G. Kristinsson. Marita collected and analysed data from records in Faroe Islands, Iceland and Denmark. Marita wrote the manuscript with supervision of Karl and co-authors Thorolfur Gudnason, Ulrich Stab Jensen and Niels Frimodt-Møller.

Paper II: This study investigated antibacterial resistance of *Streptococcus pyogenes* (GAS) associated with antibacterial sales in the Faroe Islands. It was initiated by Marita and Karl. Marita collected samples in collaboration with 12 GPs in the

Faroe Islands. Marita and Karl planned and analysed the data. Laboratory analysis was done by Marita. They wrote the manuscript together with Shahin Gaini and Hannes Gislason. Hannes Gislason planned the statistical analyses together with Marita.

Paper III: This study investigated antibacterial resistance among *E. coli* isolates from community acquired UTIs in the Faroe Islands, Iceland and Denmark, associated with antibacterial sales. It was planned together with Karl. Data collection and laboratory analysis was performed by Marita. Data analyses was done by Marita and Karl. Shahin Gaini, supervised and wrote the manuscript, together with Marita, Karl and Hannes. Hannes Gislason, planned and did the statistic together with Marita.

Paper IV: This study investigated antibacterial resistance among *Streptococcus pneumoniae,* carriage and serotype prevalence. It was planned by Marita, Karl and Helga. Data collection was performed by Marita, Elna Krosstein and Sunneva Petersen. Laboratory analysis and serotyping was performed by Marita and Helga. Analysing of data was done by Marita, Karl and Helga. Statistical advice was given by Hjálmar Hátún. The manuscript was written by Marita, Karl, Helga, Thorolfur and Shahin.

1 Introduction

Antibacterial resistance has become one of the major global threats to public health, and new resistant pathogens are continuously emerging. The consequences of infection with resistant bacteria can be severe, including long-term illnesses, increased mortality, prolonged stays in hospitals, loss of protection during surgery and chemotherapy, all of which lead to increased costs for the health sector. Although the relationship between antibacterial consumption and antibacterial resistance is not always direct and obvious, there is little doubt that antibacterial consumption is a main reason for the world wide increasing antibacterial resistance (Bell et al., 2014; Bronzwaer et al., 2002; Goossens et al., 2005).

This thesis is based on research done in the Faroe Islands. Our focus is antibacterial consumption and antibacterial resistance in the Faroese community compared to the conditions in Iceland and Denmark. Iceland was chosen as a comparative country due to its proximity; geographically, culturally and ethnically. Denmark was chosen due to political factors, since the Faroese health sector is following Danish medical guidelines and since many physicians practising in the Faroe Islands have been trained in Denmark.

1.1 The Faroe Islands

The Faroe Islands is an archipelago consisting of 18 islands located between the Norwegian Sea and the North Atlantic Ocean, about halfway between Iceland and Norway (Figure 1). It is a self-governing country under the sovereignty of Denmark, with a small and unique gene-pooled population of just over 50 thousand inhabitants. Almost half of the population live in the capital, Tórshavn.

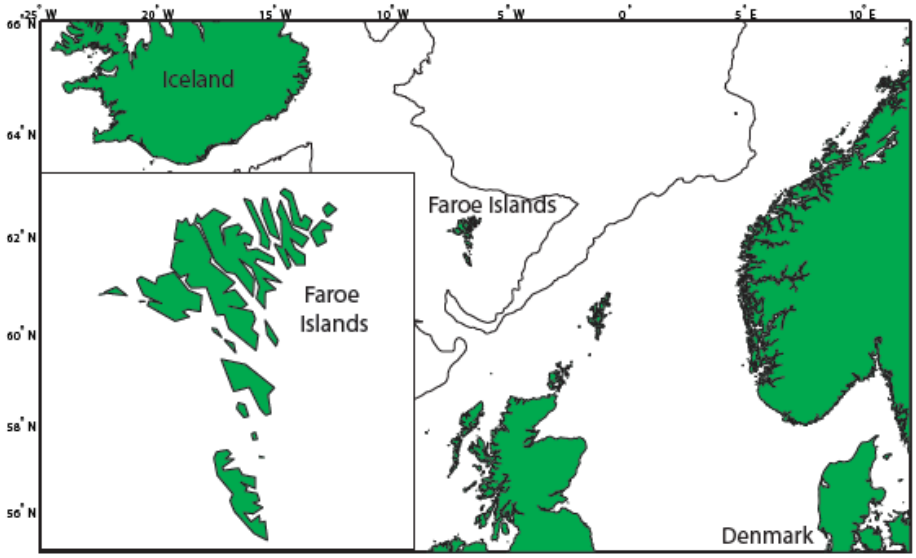

Figure 1. Location of the Faroe Islands and a close-up of its 18 islands.

Considering the global trend in increasing antimicrobial resistance, we can speculate that increased resistance might also be expected in the Faroe Islands. The threat might even be more severe than in larger countries, since resistant bacteria might emerge quickly in such a small and relatively isolated island group. Despite this, the research concerning surveillance of

antibacterial resistance and antibacterial consumption has previously been limited. In collaboration with the Danish Programme for surveillance of antimicrobial consumption and resistance in bacteria from animals, food and humans (Danmap) and Staten Serum Institut in Denmark, the National Hospital in Tórshavn established its own local surveillance database in 2011. In addition, the National Pharmacist has a database recording antibacterial prescriptions. Although the database is not publically available, the National Pharmacist has been reporting to NOMESCO for several years.

The geographic remoteness and small population of the Faroe Islands makes the archipelago an ideal location for carriage, clonal, and vaccine studies. Rapid development of herd protection is likely here, since the populations in the rural areas and Tórshavn are in frequent contact, and many people in the rural areas work in Tórshavn. Therefore, it is realistic to make comprehensive surveys of an entire nation – which is a rare opportunity.

Here we utilize these advantages and comprehensive surveys of the Faroese community to approach the critical question of antibacterial resistance. Our research is the first of its kind performed on the Faroe Islands, and therefore the data we present regarding antibacterial sales and antibacterial resistance in three human bacterial pathogens, *Streptococcus pneumoniae* (pneumococci), *Streptococcus pyogenes* (GAS) and *Escherichia coli* (*E. coli*) is entirely novel.

1.2 Antibacterial resistance

Antibiotics, also known as antimicrobial drugs, are substances that kill or interfere with the growth of micro-organisms, especially bacteria. After discovery of antibiotics in the 1940s, these compounds transformed medical care and dramatically reduced illness and death from infectious diseases. However, throughout the following decades, several bacteria that antibiotics formerly could control developed resistance to these drugs. Antibacterial resistance occurs when bacteria change in a way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections, or they may have inborn mechanisms, such as efflux pumps, beta-lactamase production etc. The bacteria survive drug treatment, continue to multiply and may thus cause more harm. The spread of antibiotic-resistant bacteria in the environment depends on the presence and transfer of resistance genes among microorganisms, mutations, and selection pressure to keep these genes in a population. Widespread use of antibiotics promotes the spread of antibiotic resistance (Centers for Disease Control and Prevention, 2013; WHO, 2017; 'WHO | Global action plan on antimicrobial resistance', 2017).

After the European Union recognised this threat, it has been recommended that European countries should collect data on antibacterial consumption and synchronize research on antibacterial resistance (The Council of the European Union, 2001).

Today, virtually all major bacterial pathogens throughout the world are becoming resistant against one or several antibiotics. Antibacterial consumption is increasing in many countries, and a clear correlation between antibacterial use and the development of resistance has been identified (Austin et al., 1999; WHO, 2005; “WHO | Global action plan on antimicrobial resistance,” 2017).

Antibiotic resistance can result in more severe illness and might also pose a greater risk of death. Alternative available antibiotics may be less effective, cause more side effects, and are often more expensive than conventional drugs.

Problems associated with the presence of antibiotic-resistant bacteria have reached epidemic proportions in recent years, with the estimated of costs associated with antibacterial resistance estimated to be $21-34 billion dollars in the United States alone (WHO, 2014). The causes and effects of antibiotic overuse are varied. One of the most controversial applications of antibiotics, however, is for growth promotion in livestock, and this application has raised concerns about its contribution to the presence of resistant bacteria in humans (Thorsteinsdottir et al., 2010; Founou et al., 2016; Manyi-LoH et al., 2018).

Because of the overwhelming threat of antibacterial resistance in community-acquired infections, the World Health Organisation (WHO) has advised to limit the use of antimicrobials to treatment of established bacterial infections, and to use as narrow spectrum of antimicrobials as possible in most instances (WHO, 2014).

There is a variation in antibacterial resistance prevalence in

Europe, depending on bacterial species, antimicrobial group and geographical region (European Centre for Disease Prevention and Control, 2016). In southern and central Europe the antibacterial resistance is very high, while it remains low in northern Europe (Goossens et al., 2005; Van de Sande-Bruinsma et al., 2008).

1.3 Antibacterial sales in Nordic countries

In Nordic countries, the clear majority of human consumption of antibiotics occurs in the community. In the Faroe Islands, antibiotics are available only by prescription and the antibiotic consumption is comparable to the consumption in Denmark (Figure 2).

The mean use of outpatient J01 antibiotics in 1997-2009 was 13.9, 15.1, 15.6 and 20.9 defined daily dosage (DDD)/1000 inhabitants/day (DID) in Denmark, Sweden, Norway and Iceland, respectively (Adriaenssens et al., 2011a). Narrow-spectrum penicillin is the most commonly used group in Denmark, Sweden and Norway, whereas broad-spectrum penicillin and combination of penicillin's, incl. ß-lactamase inhibitors are the most used sub-groups in Iceland (Versporten et al., 2011). The main difference between J01 antibacterial consumption in Denmark and Iceland, is the high consumption of tetracycline in Iceland (Coenen et al., 2011) (Figures 2 & 3).

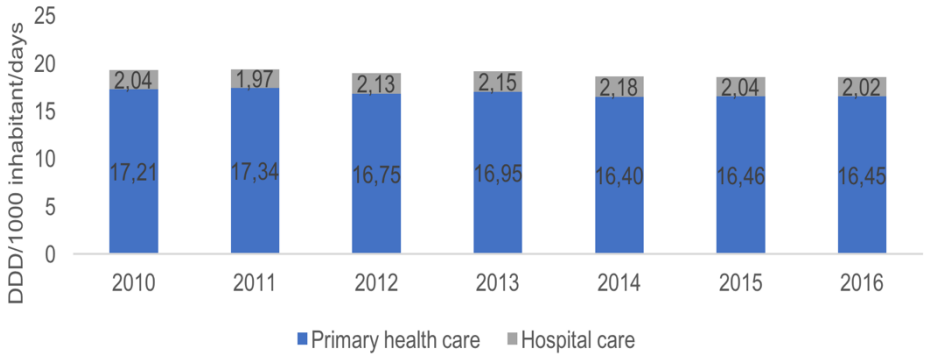

Figure 2. Total consumption of systemic antimicrobial agents (J01) in humans in Denmark, 2010-2016 (Danmap, 2016).

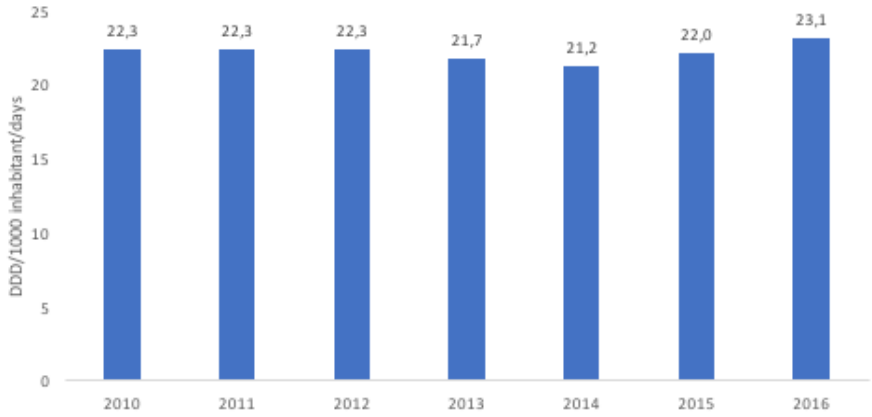

Figure 3. Total consumption of systemic antimicrobial agents (J01) in humans in Iceland, 2010-2016 (Directorate of Health, Iceland, 2016).

1.4 *Streptococcus pneumoniae*

Worldwide, *Streptococcus pneumoniae* (pneumococci) infections are a major cause of mortality and morbidity. In 2005, the WHO estimated 1.6 million global deaths annually with 0.7 to 1 millions

of those cases being children under the age of five years (WHO, 2005). In Europe and the United States pneumococci are estimated to cause approximately 30% to 50% of community-acquired pneumonia requiring hospitalization in adults (WHO, 2012).

Pneumococcal carriage and disease

The natural habitat of *S. pneumoniae* is the upper respiratory tract of humans, mainly the nasopharynx, and the pneumococci can be carried for a period of weeks to months (Loda et al., 1975). In most cases carriage does not lead to pneumococcal disease and therefore it can be considered as a part of the normal flora in the upper respiratory tract of individuals at some point, with acquisition and carriage being highest in children during the first two years of life (Kristinsson, 1997; Syrjanen et al., 2001; Bogaert et al., 2004). One of the strongest risk factors for infants, is the presence of pneumococcal carriage in an older sibling (Leino et al., 2001), and children between age 2 and 5 years are probably the main sources of pneumococcal transmission. The carriage occurrence varies, but many studies suggest an increase during first year of life (Gray et al., 1982; Aniansson et al., 1992; Dagan et al., 1996; Syrjanen et al., 2001), and a subsequent decrease after children reach the age of four (Leino et al., 2001). Other factors contributing to increased pneumococcal carriage can be the cold winter months, which was recognized as a factor early in the 1900s (Gray et al., 1982). Several studies have identified day care centres (DCCs) as a significant risk factor for pneumococcal

carriage (Yagupsky et al., 1998; Kellner & Ford-Jones, 1999; Arason et al., 2006). Children attending DCC are at a higher risk of carrying PNSP and other resistant pneumococcal strains (Henderson et al., 1988; Duchin et al., 1995; Arason et al., 1996; Yagupsky et al., 1998). For this reason, children attending DCCs often acquire respiratory tract infection, otitis media (OM) and pneumonia and account for many of the prescriptions for antibiotics (Thrane et al., 2001).

Pneumococcus along with non-typeable *Haemophilus influenzae* are the main bacterial causes of acute otitis media (AOM) (Leibovitz and Greenberg 2004) and studies verify that there is an association with pneumococcal carriage and OM (Zenni et al., 1995). Approximately 60% of all children experience one or more episodes of AOM during the first year of life (Kilpi et al., 2001). OM is also responsible for most of their visits to health care (Teele et al., 1983) and for most of the use of antibacterial agents (Arason et al., 1996). Invasive pneumococcal disease (IPD), for example sepsis, meningitis, and respiratory tract infections are age-dependent, with the greatest incidence occurring among the very young and the elderly (Bryce et al., 2005). IPDs are the most severe pneumococcal infections and always require antibacterial treatment. IPDs are the main rationale supporting pneumococcal vaccination (WHO, 2007).

The mean mortality in relation to IPD is 18% in Denmark, with approximately 190 deaths annually (Harboe et al., 2008). Incidence of IPD in the Faroe Islands is perceived to be low when

compared to Denmark or Iceland (Harboe et al., 2008; Hjálmarsdóttir et al., 2017). A likely explanation for this could be the clinical practices and the criteria for performing blood cultures from patients in the Faroe Islands differ from that in the neighboring countires.

Antibacterial susceptibility

Since sulphonamides were introduced in the 1930s to treat infections, several antibiotics with different treatment effects have been developed.

Penicillin was discovered by Alexander Fleming in 1928, but not used for treatment of infection until 1941 when Florey's team used penicillin to treat a patient with a bacterial infection (Fletcher 1984). Penicillin, and the other antimicrobials that followed rapidly thereafter, were a therapeutic revolution for treatment of lethal infections as well as many chronic and disabling infections.

Since the early 1970s, PNSP have spread globally and become common in many areas (Forward, 1999). Antibacterial consumption has been identified as the main risk factor for their spread (Pallares et al., 1987; Arason et al., 1996; Kristinsson 1997). Other factors such as prior hospitalization (Pallares et al., 1987), young age (Hofmann et al., 1995) and DCCs attendance (Duchin et al., 1995; Fairchok et al., 1996) also contribute to increasing resistance. The emergence of resistance to penicillin and other beta-lactam antibiotics in pneumococci in the 1980s and 1990s led to the increased use of macrolides, fluoroquinolones and other non-beta-lactam antibiotics for

pneumococcal infections (Tuomanen et al., 2004). The overall percentages of *S. pneumonia* strains with non-susceptibility to commonly used antibacterials reported to EARS-Net have remained relatively stable in Europe during recent years. The lowest percentages with non-susceptibility to penicillin and macrolides were reported in central and northern Europe (ERAS, 2011). In 2015, a wide variation between European countries was recorded. Macrolide non-susceptibility in *S. pneumoniae* was, for most countries, higher than penicillin non-susceptibility (ERAS, 2015).

Of special concern during the last decade is the increasing prevalence of macrolide-resistant pneumococci in community-acquired infections. Comparative and analytical studies have demonstrated a relationship between the use of antibacterials and increase in bacterial resistance to these drugs in the community (Molstad et al., 1988; Arason et al., 1996; Seppala et al., 1997; Arason et al., 2002; Arason et al., 2006).

Although antibacterial consumption is mainly linked to antibacterial resistance, international multi-resistant clones are now acknowledged as an important factor in spreading PNSP (Soares et al., 1993; Arason et al., 2002).

Resistance trends in Iceland and Denmark

Antibacterial treatment of AOM is debatable. However, the Faroe Island are following the Danish medical guidelines, which recommend penicillin as the first-line treatment for pneumococcal disease and macrolides for patients with penicillin allergies. In the

Faroe Islands, erythromycin is the drug of choice within the macrolides class.

In Iceland, the percentage of *S. pneumoniae* isolates sent to the Microbiological Department, Landspitali University Hospital that were non-susceptible to penicillin increased from 2008 to 2012 (38% to 42%) followed by a decrease during the period from 2012 to 2016 (42% to 22%) (Hjálmarsdóttir et al., 2017). The non-susceptibility to erythromycin increased from 2008 to 2011 (39% to 45%) and decreased from 2011 to 2015 (45% to 23%) and then increased again in 2016 to 33%. Tetracycline had the same trend as penicillin resistance (Landspítali, 2006-2016). The percentage of *S. pneumoniae* invasive isolates that were non-susceptible (resistant and intermediary resistant) to penicillin varied with little change from 3.6% in 2009 to 6.2% in 2016 in Denmark. This was similar to the levels reported to EARS-Net by other Scandinavian countries, such as Norway and Sweden (Danmap, 2008-2016). Macrolide non-susceptibility in pneumococcal isolates from blood and cerebrospinal fluid also varied minimally from 3.6% in 2009 to 4.8% in 2016. The level of erythromycin non-susceptibility in Denmark was similar to the level in Sweden in 2015 (6.6%) but lower than in Norway (10.7%) (Danmap, 2008-2016).

Pneumococcal carriage research in Iceland is well established with studies since 1992. In the period 1992 to 1999, the PNPSs prevalence was 13.9% (Tomasson et al., 2005). Followed by a decrease and thereafter an increase in 1995 to 2010, this variation was explained by several factors, circulation of

multiresistant clones and antibacterial use (Hjálmarsdóttir & Kristinsson, 2014).

A study from 2014, showed a steady prevalence among PNPSs in pneumococcal carriage study in the years 2009 to 2013 (Hauksson et al., 2014).

During the research in Iceland features of the multiresistant clones have been identified. Clones like Spain^{23F}-1, Spain^{6B} -2, Portugal^{19F}-21, Greece^{6B}22 and Colombia^{23F}-26 have been circulating in Iceland (Soares et al., 1993; Kristinsson, 1995; Sa-Leao et al., 2000; Sa-Leao et al., 2002) and despite a decrease in antimicrobial use the resistance level of PNSP has not decreased (Arason et al., 2002; Siira et al., 2009; Hjálmarsdóttir & Kristinsson, 2014). However, a study conducted from 2009 to 2015 showed a decrease in resistance prevalence's after the introduction of the 10-valent protein conjugated pneumococcal vaccine (PHiDCV) in 2011 (Sigurdsson et al., 2017).

Serotypes

Pneumococci are enclosed within a capsule consisting of repeated units of polysaccharides. The composition of the polysaccharides defines the serotype. In general, the prevalence of pneumococcal serotypes causing IPD may be diverse due to differences in blood culture practices, geographic area, age and time (Scott et al., 1996). Approximately 100 serotypes have been recognised, while the most invasive *S. pneumoniae* infection are caused by a small number of serotypes. The serotypes typically carried by healthy children, such as 6A, 6B, 14, 19F and 23F, are

considered to have a low invasive potential. Those serotypes with a high invasive potential are rarely seen in carriage (Brueggemann et al., 2003; Hjaltested et al., 2003; Tomasson et al., 2005).

In adults, types 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 11A, 12F, 14, 18C, 19A, 19F, 22F and 23F are predominant and are covered in the 23-valent vaccine (Henriques et al., 2000). In children types 1, 4, 5, 6A, 6B, 9V, 14, 18C, 19A, 19F and 23F have been among the predominant types causing invasive disease (Center for Disease Control and prevention (CDC) 1997). Serotype 3 occurs frequently in AOM but rarely causes invasive disease in children (Tuomanen et al,. 2004). In Europe the serotypes causing invasive infection are mainly serotypes 14, 1, 7F, 3, 6B and 23F (Nielsen & Henrichsen, 1992). In Sweden serotypes 14, 7F, 9V, 3, 6B and 23F were the most common (Hedlund et al., 1995). Serogroups 6, 14 and 19 are among the leading causes of invasive infection among young children in all region of the world (Hausdorff et al., 2000).

Pneumococcal Vaccines

In 2008, the pneumococcal vaccine program was initiated in the Faroe Islands with protein conjugated vaccines, Prevenar/Prevnar (Pfizer, Philadelphia, USA) PCV-7. PCV-7 includes purified polysaccharides of 7 serotypes, the serotypes, 4, 6B, 9V, 14, 18C, 19F and 23F. In early 2010, PCV-7 was replaced with PCV-13. PCV-13, Prevenar 13 (Pfizer, Philadelphia, USA) includes the polysaccharides of 13 serotypes,

the serotypes, 4, 6B, 9V 14, 18C, 19F, 23F, 1, 5, 7F, 3, 6A and 19A. The vaccine is given at ages 3, 5 and 12 months.

Pneumococcal polysaccharide vaccines have generally minimal or no effect on pneumococcal carriage prevalence (Dagan et al., 1996; Sigurdsson et al., 2017). However, several studies have demonstrated a reduction in vaccine serotypes and increase in occurrence on non-vaccine serotypes after introduction of pneumococcal polysaccharide vaccines (Eskola et al., 2001; Sa-Leao et al., 2009; Harboe et al., 2012; Nakano et al., 2016; Sigurdsson et al., 2017).

1.5 *Streptococcus pyogenes* (group A streptococcus, GAS)

Carriage and disease

GAS are Gram-positive cocci. Although some individuals are asymptotic carriers, GAS are usually not a part of the normal flora of humans (Bartelt, 2000).

GAS can be divided into more than 100 M-serotypes or *emm* types based on the M protein. Their virulence is related directly to the M protein on the cell surface that inhibits phagocytosis. Infections caused by GAS include pharyngitis, impetigo, pyoderma, cellulitis, myositis, necrotizing fasciitis, osteomyelitis septic arthritis, bacteraemia, toxic shock syndrome and pneumonia (Smith, 2005; Martin & Green, 2006). GAS lives only in humans, and are not found anywhere else in nature. They are

generally found in the nasopharyngeal passages and occasionally on the skin.

GAS is commonly transmitted through direct person to person contact. They can also spread through air via fluid droplets leaving the nose or throat. Due to the spread from person to person, the peak incidence of infection with the bacteria occurs during the winter months when people are most often indoors (Smith, 2005), and sneezing and coughing due to the common cold. Parents of school-aged children and adults who are in contact with children will have higher risk for strep throat than adults who are not around children (Center for Disease Control and prevention (CDC), 2016).

The global burden of GAS is estimated to cause 517.000 deaths each year due to severe GAS diseases (Cararpetis et al., 2005).

Antibacterial susceptibility

At present penicillin or amoxicillin is considered the first-line therapy in treatment of most GAS infections (Shulman et al., 2012). Macrolides such as erythromycin have been most commonly used for the treatment of GAS infections, especially for those who are allergic to penicillin. The Faroe Islands follow the Danish medical guidelines which recommends penicillin and macrolides for patients with penicillin allergy for treatment of tonsillitis caused by GAS. Erythromycin is the primary sub-group used in the Faroe Islands.

GAS remains uniformly susceptible to penicillin (CDC, 2016). The emergence of macrolide resistance is however worrisome and is a global challenge (Kataja et al., 1999; Reinert et al., 2004; Gattringer et al., 2004) . Resistance prevalence's from 2% to 98% have been reported for macrolides worldwide. In several European countries, an increase of macrolide resistance has been described during the last 10-20 years (Silva-Costa et al., 2015). However, in recent years there has been a decline in macrolide resistance in Italy and Portugal (Gherardi et al., 2015; Silva-Costa et al., 2015).

There are many factors contributing to antimicrobial non-susceptibility, including antibacterial consumption. However, the circulation of clones can also be responsible for variations in the prevalence of antibacterial resistance (Seppala et al., 1997; Silva-Costa et al., 2006; Silva-Costa et al., 2008) . In Sweden in 1987 there was a connection in resistance spread within and between two families and one DCC (Zackrisson et al., 1988).

Resistance trends in Iceland and Denmark

GAS isolates remain susceptible to penicillin in Iceland and Denmark (as everywhere else). In 2008, there was a high peak in erythromycin resistance in Iceland (45%) and a decrease in 2009 (12%), however, erythromycin non-susceptibility of GAS has remained stable since 2009 to 2016. Clindamycin non-susceptibility has increased from 1% in 2009 to 4% in 2016 (Landspítali, 2006-2016).

In Denmark, non-susceptibility to erythromycin in invasive GAS

did not vary much from 2009 to 2016 (4.5%-5.2%). Variation in clindamycin non-susceptibility was minimal from 2013 to 2016 (1.7%-0.6%) (Danmap, 2008-2016).

1.6 *Escherichia coli* (*E. coli*)

General description and disease

In 1885, a German paediatrician named Theodor Escherich was isolating bacteria from diapers of healthy babies when he noticed a rod-shaped microbe that could produce, in his words, a "massive, luxurious growth". It flourished on all manner of food and blood. In 1969 the biologist Max Delbrück was awarded a Nobel Prize for his work on *E. coli* and its viruses - he stated "This riddle of life had been solved" (Zimmer, 2008).

E. coli is a part of the normal intestinal microbiota in humans and a common cause of bacterial infections. Most *E. coli* are harmless and represent an important part of a healthy human intestinal tract.

Urinary tract infections (UTIs) are among the most common bacterial infection handled in primary care and *E. coli* is the most common pathogen causing UTIs (Olafsson et al., 2000; Kahlmeter, 2003; Ronald, 2003; Schito et al., 2009).

E. coli is the most frequent Gram-negative bacillus isolated from blood cultures in clinical settings. It is the most frequent cause of bacteraemia, community and hospital-acquired UTIs. It is associated with spontaneous and surgical peritonitis and with

skin and soft tissue infection due to multiple micro-organisms, causes neonatal meningitis and is one of the leading causative agents in food-born infections worldwide (ECDC, 2016).

E. coli can acquire antimicrobial resitance as a consequence of antibiotic use in agriculture and selective pressures, and it has the potential for transferring resistance to pathogenic bacteria and can cause infection in humans (Danmap, 2016). Moreover, human travel allows bacterial plasmids and clones to be transported rapidly between countires (Kumarasamy et al., 2010).

Antibacterial treatment in the Faroe Islands

Trimethoprim is recommended as the empirical treatment of choice for uncomplicated UTIs in several European countries (Christiaens et al., 2004). However, in most Nordic countries pivmecillinam and trimethoprim are among the first-line antibiotics used for treatment of UTIs. In the Faroe Islands sulfamethizol is also used routinely, due to the high prevalence of a rare genetic disease, primary carnitine deficiency (PCD). The use of pivmecillinam and pivampicillin is thus contraindicated because it increases the risk for arrhythmia and cardiac arrest in PCD patients (Rasmussen et al., 2012).

Antibacterial susceptibility

In the Faroe Islands, many GPs perform urine culturing on-site and thus only submit urine samples for culturing at the central diagnostic laboratory located at National Hospital in Tórshavn in case of e.g. treatment failure or difficulty interpreting of their on-

site acquired results from susceptibility testing. Hence there are no surveillance data in the Faroe Islands regarding susceptibility testing in *E. coli*.

WHO has listed *E. coli* as bacteria of international concern, due to *E. coli* strains resistant to third-generation cephalosporins, with extended spectrum ß-lactamases (ESBLs), and resistant to fluoroquinolones (Chaudhary, 2016). EARS-Net reported in 2015 that more than half of the *E. coli* isolates were resistant to at least one antimicrobial group under surveillance. The most frequent were aminopenicillin and fluoroquinolones, with resistance to both aminoglycosides and third-generation cephalosporins increasing between 2012 and 2015. Carbapenem resistance is still rare in Europe, however, the highest resistance percentages were reported from southern and south-eastern Europe (ECDC, 2015a).

The ECO-SENS project study published by (Kahlmeter, 2003) showed that *E. coli* in UTIs have high resistance to ampicillin and sulfamethoxazole, trimethoprim and trimethoprim-sulfamethoxazole (SXT) and the resistance prevalence was low to mecillinam, amoxicillin/clavulanic acid (AMC), nitrofurantoin and fosfomycin in Europe. However, non-susceptibility to fluoroquinolones among *E. coli* in Europe varied.

The relationship between antibacterial sale and resistance among *E. coli* requires a better understanding. Mathematical modelling provide some insight (Austin et al., 1999), and suggests a need for well-designed studies to investigate the relationship

between antibacterial sale and resistance. In our *E. coli* study (Paper III), we attempt to demonstrate a model that shows slow and fast development resistance among *E. coli* isolates in Faroe Islands, Iceland and Denmark.

Resistance and *E. coli* producing carbapenemases and plasmid-mediated colistin resistance

Gram-negative bacteria increase their multi-resistance faster than in Gram-positive bacteria (Cornaglia, 2009) and there are few new developed antibiotics against Gram-negative bacteria (Baiden et al., 2010).

Resistance in *E. coli* often develops by acquisition of mobile genetic elements encoding resistance mechanisms, such a production of extended spectrum beta-lactamases (ESBL) and carbapenemases (ECDC, 2016). A high level of resistance has been reported from food-producing animals in Europe, including carbapenemase production and plasmid-mediated colistin resistance (EFSA (European Food Safety Authority), 2017) this is worrisome and therefore a surveillance regarding the transmission of resistance between animals and humans is essential. Due to the serious problem of ESBLs, the use of reserved antibiotics such as carbapenems is necessary (Kumarasamy et al., 2010). Carbapenem resistance is an ongoing public-health problem worldwide, however carbapenem resistance in *E. coli* have not been reported in blood cultures in Iceland and Denmark (ECDC, 2016). The increasing resistance to polymyxins is also of concern, considering that polymyxins are

last-resort antibiotics for treating infections due to carbapenem resistance.

Resistance trends in Iceland and Denmark

In a study from Iceland in 1992 to 1995, the *E. coli* isolates from community acquired UTIs were non-susceptible to ampicillin in 36% of the cases and 24% to sulphonamides. Non-susceptibility to mecillinam was already 10% and, the conclusion was that a high consumption of antibiotics was the probable reason (Olafsson et al., 2000). In Iceland an increase in resistance to ciprofloxacin has been reported from 12% to 14% (2009 to 2010) and 13% to 16% in (2015 to 2016) and a decrease in mecillinam resistance from 7% to 6% from (2015 to 2016) (Jonsdottir & Kristinsson, 2008; Landspítali, 2006-2016).

The increasing prevalence of fluoroquinolone resistance among human *E. coli* isolates also coincided with the high prevalence of quinolones-resistance among *E.* coli isolates obtained from broilers and broiler meat in Iceland in 2008 (Thorsteinsdóttir et al., 2010).

In Denmark, resistance data from blood stream infections caused by *E. coli* showed a significant increase in the resistance prevalence to gentamicin (3.9% to 6.1%), and cefuroxime (5.7% to 8.6%) from 2007 to 2016. From 2007 to 2016, a significant increase of ciprofloxacin resistant *E. coli* isolates were recorded in urine cultures from primary health care. This increase happened primarily from 2007 to 2009 and resistance level has remained stable since 2009. A significant increase in mecillinam

resistance was observed from 2007 to 2016, from 3.9% to 5.6%. Even though the resistance level was high in sulphonamides and ampicillin the resistance level did decrease from 2007 to 2016 (Danmap, 2016). The prevalence of third-generation cephalosporin-resistant *E. coli* is still increasing in Europe. In Denmark, the occurrence was low before 2007, but has increased since, mostly from 2007 to 2011 (Danmap, 2016).

Iceland and Denmark are in the top six countries in Europe with the lowest resistance prevalence among invasive *E. coli* isolates. Iceland has the lowest prevalence of fluoroquinolones (9.6%) and third-generation cephalosporins (4.2%) resistance, compared to Cyprus which has 47.0% resistance prevalence in fluoroquinolones and Bulgaria which has 41.6% resistance prevalence's to third-generation cephalosporins (ECDC, 2016).

The combined resistance to fluoroquinolone, third-generation cephalosporins and aminoglycosides in both Iceland (1.1%) and Denmark (1.8%) are among the lowest in Europe, when compared to the southern countries in the southern portion of Europe (10-22%) (ECDC, 2016).

2 Aims

The aims of the studies described in the thesis were:

I. To describe, compare, and analyse the sales of J01 systemic antibacterial drugs in the Faroe Islands, Iceland and Denmark in 1999-2011.

II. To investigate the prevalence of antibacterial resistance in *Streptococcus pyogenes* (GAS) isolates in the Faroe Islands, from patients with tonsillitis and carriers (children), compare the findings with similar data in Iceland and correlate the antibacterial resistance to antibacterial sales.

III. To examine the antibacterial resistance among *E. coli* isolates from community-acquired UTIs, and determine if antibacterial resistance prevalence correlates with antibacterial sales in the Faroe Islands, Iceland and Denmark.

IV. To describe and compare carriage, antibacterial resistance and serotype prevalence of *Streptococcus pneumoniae* in healthy Faroese children aged 0-7 years

old attending DDCs in the period 2009-2011. To investigate antibacterial resistance and serotype prevalence among invasive *Streptococcus pneumoniae* in the Faroe Islands from 1994-2016, and to compare our findings with data from Iceland.

3 Material and methods

This thesis is based on four studies. Paper I describes the sale of antibacterial agents in the Faroe Islands, Iceland and Denmark within the period 1999-2011. To complete the analysis, three cohort studies were performed by collecting bacterial isolates, followed by investigating antibacterial resistance, pneumococcal carriage and serotype prevalence in the Faroese community.

Paper II is based on collecting GAS in the Faroese community and investigating antibacterial resistance in the isolates and correlating the data on resistance with antibacterial sales.

Paper III is based on collecting samples from patients with community acquired UTIs, and investigating the antibacterial resistance in *E. coli* and then analysing the association between *E. coli* resistance and antibacterial sales on the Faroe Islands.

Paper IV was conducted at 30 DCCs in order to describe pneumococcal carriage, antibacterial resistance and serotype prevalence among carried pneumococci and among invasive pneumococcal isolates.

3.1 Study population

This study aimed to focus on the 18 islands (geographic regions) which comprise the Faroe Islands. However, some of the islands are so small they do not have DCCs and if the inhabitants need medical care, they must travel to a larger island nearby.

3.1.1 Antibacterial Sales (paper I)

For the Faroe Islands, data on antibacterial sales was collected from the National Pharmacist in the Faroe Islands. Data from Iceland and Denmark was collected from the Icelandic Medicine Agency and Nordic Medico-Statistical Committee (NOMESCO) and Danmap (DANMAP, 2008-2016; Lyfjastofnun, 2011.; Nomesco, 2004).

Collected data represented the total use of systemic antibacterials (ATC J01) expressed in (DDD)/1000 inhabitants/day (DID) after the WHO guidelines for ATC classifications and DDD assignments (WHO, 2011).

The total antibacterial sale was described for all the major group ATC classifications for 1999-2011. Tetracycline J01A, penicillins J01C, cephalosporins J01D, sulphonamides and trimethoprim J01E, macrolides and lincosamids J01F, aminoglycosides J01G, quinolones J01M and other antibiotics J01X. Age-specific data on the use of macrolides in the community was based on outpatient prescriptions.

3.1.2 Patients (papers II and III)

The patient samples were routine clinical oropharynx and urine samples collected by 12 GPs in the Faroe Islands. The GPs were selected with the intent to represent all the 18 islands. All the patients were eligible for participation after informed consent was obtained from them or their parents (children) (see Appendix II). The sampling period was 2009 to 2010 (January to March) and in 2012. During this period, the GPs sampled 125 oropharyngeal and 210 urine samples.

3.1.3 Healthy children (papers II and IV)

Nasopharyngeal swabs were collected from healthy children aged 0-7 years attending 30 DCCs in the Faroe Islands during January to March 2009, 2010 and 2011. During this period, pneumococcal isolates were collected. GAS isolates, were also cultured in this study. They were collected by the two same persons, two qualified nurses for the length of the study. Normally, children are enrolled in DCC in August in the year they turn one year old and they leave the DCC when they become 6-7 years old to begin primary school. Children aged ≤ 7 years represented about 11% of the population of the Faroe Islands (Hagstova Føroya, 2016).

All the children were eligible for participation after informed consent was obtained from their parents or legal guardian. They answered a questionnaire on the use of antibiotics, vaccinations,

number of siblings under 6 years and the occurrence of otitis media in their child (see Appendix II).

3.1.4 Invasive isolates (paper IV)

Information about age, sex, susceptibility testing, serotype and origin of the pneumococcal isolates of invasive pneumococci isolates from 1974 to 2016 was obtained from the Statens Serum Institut (SSI, Copenhagen, Denmark). There were 60 pneumococcal isolates from blood (*n* = 52) and spinal fluid (*n* = 8). Before 1978 all microbiological samples were sent to SSI for routine diagnostics. A local laboratory of clinical microbiology at the National Hospital Faroe Islands was established in 1978. Blood cultures were done in the Faroe Islands from 1978 to 1999 using conventional blood culture bottles, but after 1999, using the BacTec blood culturing platform. The exact number of invasive pneumococci before 2007 is not known. Since 2007, all invasive pneumococci have been sent to SSI as a part of the mandatory national surveillance of pneumococcal serotypes in the Kingdom of Denmark including the Faroe Islands.

3.2 Isolation and identification

3.2.1 Laboratory Procedures

3.2.1.1 Oropharyngeal and nasopharyngeal swabs (papers II and IV)

The oropharyngeal samples were obtained with a sterile charcoal medical applicator and the nasopharyngeal samples with a sterile

eswab, Coban ® (Transport medium swabs, Copan, Italy). The samples were stored for a maximum of 24 hours in transport medium, before inoculation on blood agar containing gentamicin (5 mg/L). Plates inoculated with nasopharyngeal swabs contained an optochin disc (Oxoid, UK) while plates inoculated with oropharyngeal swabs contained a bacitracin disc (Oxoid, Roskilde, Denmark) located in the centre of the plate. Following inoculation, agar plates were incubated anaerobically at 35ºC for 18-24 hours.

Oropharyngeal swabs: Colonies with characteristic appearance were identified as *Streptococcus pyogenes* (GAS) by bacitracin disc sensitivity and grouped by Lancefield grouping (ImmuLex^{TM} Streptococcus Group Kit).

Nasopharyngeal swabs: Colonies with characteristic appearance were identified as *Streptococcus pneumoniae* by optochin disc sensitivity and α-haemolysis and confirmed by susceptibility to optochin incubated in 5% CO_2 at 35ºC for 18-24 hours.

3.2.1.2 Urine samples (paper III)

Mid-stream urine samples from patients in all age groups whose urine was positive in a dip strip test for leukocytes, nitrate, or both were deposited on a dipslide containing CLED and MacConkey agar at the GPs clinic. Within 24 hours, dipslides were incubated under aerobic condition at 35ºC for 18-24 hours. Colonies with a minimum count of 100.000/mL were taken from the MacConkey

agar and inoculated on 5% sheep blood medium and incubated aerobically at 35ºC for 18-24 hours. Colonies with characteristic appearance that were oxidase negative and indole positive were considered to be *E. coli*.

3.3 Antibacterial analyses

3.3.1 Antibacterial susceptibility testing for GAS isolates (paper II)

Disc susceptibility testing was performed on all GAS isolates using the CLSI methods and criteria, which was appropriate at the given time (CLIS & Standards, 2017). Susceptibility to penicillin, erythromycin, clindamycin and tetracycline was tested with disc diffusion test.

3.3.2 Antibacterial susceptibility testing of *E. coli* (paper III)

Disc susceptibility testing was performed on all *E. coli* isolates using the CLSI methods and criteria, which was appropriate at the given time (CLSI & Standards, 1998). Susceptibility to ampicillin, cefoxitin, trimethoprim, sulfamethoxazole/trimethoprim, nitrofurantoin, mecillinam, cefpodoxime, amoxicillin/clavulanic acid, cefuroxime, ceftriaxone, nalidixic acid, gentamicin, ciprofloxacin and sulphonamide (sulfamethizol) was performed with disc diffusion test.

3.3.3 Antibacterial susceptibility testing of pneumococci (paper IV)

All pneumococcal isolates were tested for antibacterial susceptibilities using disc diffusion and the EUCAST methods and criteria (www.eucast.org) to erythromycin, clindamycin, tetracycline, chloramphenicol and trimethoprim-/sulfamethoxazole. The isolates were screened for penicillin non-susceptibility with 1 µg oxacillin discs and penicillin MIC measured for all oxacillin resistant isolates using E-test (BioMerieux, France). Isolates with MIC ≤ 0.064 mg/L to penicillin were considered fully susceptible to penicillin and isolates with > 0.064 mg/L were defined as PNSP.

3.4 Serotyping

3.4.1 Serotyping (paper IV)

All pneumococcal isolates were serotyped after conventional methods, with Pneumococcal Latex antisera, which is based on agglutination of serotypes (Slotved et al., 2004).

3.5 Statistical analysis

The statistical analysis was made by using the R-language, which is a programming language and software environment for statistical analysis for statistical computing and graphics (www.r-project.org) and the RStudio integrated development

environment (www.rstudio.com). Statistical analyses were also performed in Matlab (https://www.mathworks.com).

3.5.1 Statistical methods (paper II)

To evaluate difference in resistance in GAS isolates between the sampling years and to associate tetracycline resistance to age group, Fisher's exact test was used, *p-value* ≤ 0.05 was considered significant.

3.5.2 Statistical methods (paper III)

To test for a significant difference between *E. coli* resistance in the years 2009/2010 and 2012 in the Faroe Islands Fisher's exact test was used. To evaluate a trend in antibacterial resistance in the Faroe Islands, trend in the proportion was tested by using *prop.trend.test* as a weighted linear regression. The test was used to determine if there was no trend (zero slope) in resistance compared with sales.

To investigate the relationship between antibacterial resistance and antibacterial sales, a logistic model with modified overdispersion of antibacterial resistance with sales in the Faroe Islands, Iceland and Denmark was performed, demonstrating a possible development in resistance trends.

3.5.3 Statistical methods (paper IV)

Univariate analysis was performed using Chi-square test with df = 2 < 5.991 and z-score with *p-value* ≤ 0.05 was considered significant.

3.6 Ethics

Ethical approval for the study was obtained from the Scientific Ethical Board of the Faroe Islands on the 7th of November in 2008 and the data collection approval was obtained from the Data Protection Agency on the 6th of June in 2008.

4 Results and Discussion

The four papers which comprise this PhD thesis, are presented in chronological order by publication date. For each paper, a summary is given first, followed by an elaboration and discussion of the results. The summaries are included to highlight the common themes between the four papers and provide a clear understanding for the reader. The following results section contain both the material presented in each paper supplemented by information, data and knowledge, gained since the publication of the studies.

4.1 Antibacterial use in the Faroe Islands, Iceland and Denmark 1999-2011 (paper I)

Antibacterial use (J01) in the Faroe Islands, Iceland and Denmark, 1999-2011 was described in paper I.

4.1.1 Summary

No detailed information on antimicrobial use in the Faroe Islands had been published before our study. So, as a basis for the subsequent studies on antibacterial resistance (Papers II-IV), groundwork was needed in order to produce data on the antimicrobial use - here proxied by sales in the Faroe Islands.

Both Denmark and Iceland have collected information on antimicrobial use for a long time, but the comparison between the Faroe Islands and these ethnically and culturally related countries is novel. Important information about prescribing practices can be obtained by this comparison.

Despite the similarities between these three countries, we found marked differences in total antibacterial use and important differences in the use of individual antibacterials. Of note is the higher use in Iceland, mainly caused by the high tetracycline consumption in Iceland. The higher use of broad-spectrum penicillin and the combinations with beta-lactamase inhibitors in Iceland could be a reflection of the higher antibacterial resistance complications related to pneumococcal infections there (ERAS, 2015). We also document important differences in the use of individual penicillin's and macrolides between the countries. The antibacterial use in the Faroe Islands is relatively stable. A partial explanation for this is likely that there is no antimicrobial policy and no information on antimicrobial resistance in this country. Another possible explanation could be that most doctors practicing in the Faroe Island are trained in Denmark, compared to many Icelandic doctors are specialized in the US. One suspicion derived from this study concerns a potentially inappropriate use of erythromycin in the Faroe Islands. In order to give important information regarding the effects of community antibacterial consumption on antibacterial resistance, the differences in antimicrobial use that we report in paper I were

used in papers II-IV to analyse the relationship between antimicrobial use and antibacterial resistance data.

4.1.2 Antibacterial sales

The sales of systemic drugs subclassified as ATC J01, according to the anatomical therapeutically chemical classification system (ATC) was highest in Iceland in the period 1999 to 2011, with the mean sale 21.8 ± 0.746 DID, followed by the Faroe Islands, with mean sale 17.7 ± 0.385 DID and Denmark with the lowest mean sale 16.3 ± 1.267 DID. The sales date for J01 drugs was compiled from community, nursing homes and hospitals (Figure 4). During the period from 1999 to 2011 the antibacterial sales were stable in the Faroe Islands fluctuating between 16.3 DID in 1999 to 17.2 DID in 2011, with the highest sale in 2008 (18.4 DID). In Iceland, the antibacterial sales varied with minor fluctuations during the period 1999 to 2011, from 21.6 DID in 1999 to 22.3 DID in 2011, with the highest sale in 2006 (23.4 DID). In Denmark, the antibacterial sale steadily increased from 13.5 DID in 1999 to the highest sale in 2011 (19.5 DID) (Figure 4). This is comparable to the ESAC surveillance data (Adriaenssens et al., 2011a).

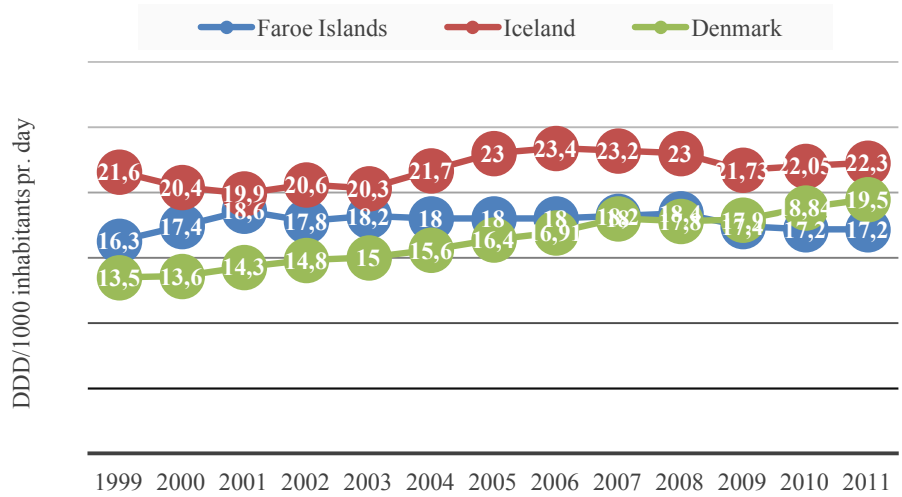

Figure 4. Total sales of antibacterial drugs for systemic use (J01), presented in defined daily doses/1000 inhabitants/day (DID) in the Faroe Islands, Iceland and Denmark, 1999-2011.

The increase in Denmark was only in the primary health care sector with an increase in four sub-groups: macrolides J01FA, combination penicillin's J01CR, penicillins with extended spectrum J01CA and beta-lactamase sensitive penicillin's J01CE. The increase was due to an outbreak of *Mycoplasma pneumoniae* in 2010 and therefore, more patients were treated in the second half of 2010. Patients with lower respiratory tract infections were empirically treated with beta-lactamase sensitive penicillins, and confirmed *M. pneumoniae* by macrolides according to guidelines in Denmark (DANMAP, 2010).

Tetracycline J01A

The most noteworthy difference between the countries was tetracycline sales; the sale was three times higher in Iceland than in the Faroe Island and Denmark in the period 2005 to 2011.

The sale of tetracycline was stable in all three countries in the period 2005 to 2011 (Figure 5). The mean sale from 2005 to 2011 of tetracycline was 1.4 DID, 1.6 DID and 5.2 DID in Faroe Islands, Denmark and Iceland, respectively. However, there was a gradual increase in the primary care sector in Denmark from 2001 to 2010 (DANMAP, 2011). This could be due to package sizes with higher numbers of tablets, and due to the traveling pattern of Danes were doxycycline is one of the recommendations in Denmark for malaria prophylaxis (DANMAP, 2008). However, the use of tetracycline has not increased in the Faroe Islands since 2008 and the Faroe Islands is following Danish medical guidelines. In Iceland, the only tetracycline used was doxycycline, which accounted for 24% of the total J01 antibacterial sale in Iceland between 2005 and 2011. Iceland had the highest use of tetracycline in Europe in 2009 (Coenen et al., 2011). From 2009 (5.1 DID) to 2016 (4.6 DID) there has been a gradual decrease in the use of tetracycline in Iceland (ECDC, 2015b). In 2015, the sale of tetracycline was 4.6 DID in the community, which was 23.1% of the total sale of the ATC group J01 (ECDC, 2015b). The tetracycline use in Iceland is comparable to the use in Finland and UK (Adriaenssens et al., 2011a). The high use in Finland may be

explained by the use of doxycycline for treatment of respiratory tract infections, (Rautakorpi et al., 1997).

In all three countries tetracycline was prescribed to teenagers and young adults with acne being the most likely indication. Why acne is treated more commonly with tetracycline in Iceland than in other countries is not known.

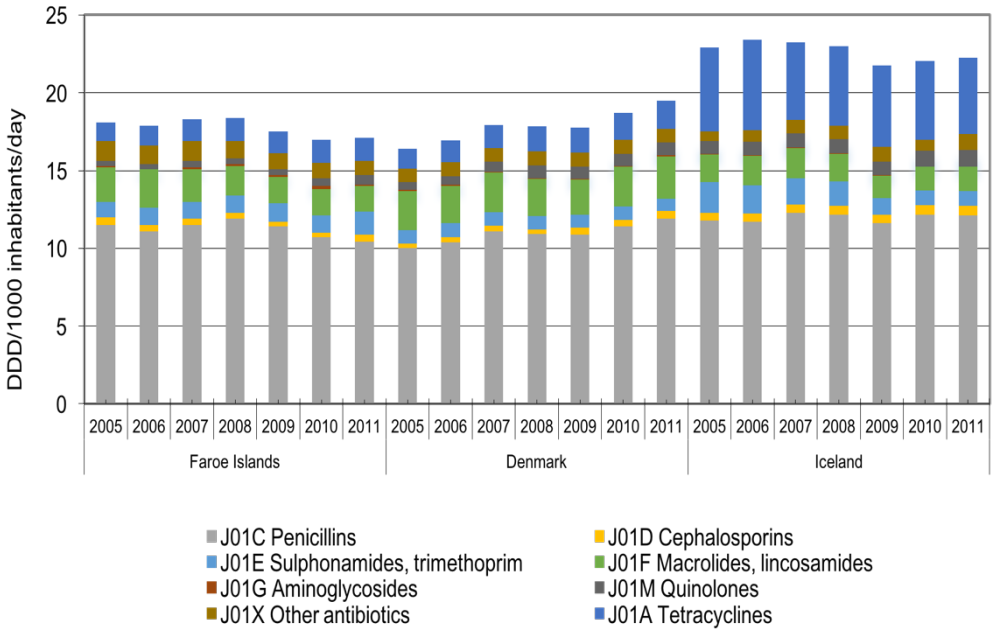

Figure 5. Sales of antibacterial groups for systemic use (ATC groups J01A, C, D, E, F, G, M and X), presented in defined daily doses/1000 inhabitants/day (DID) in the Faroe Islands, Iceland and Denmark, 2005-2011.

Penicillin J01C

As in other European countries, penicillin was the most frequently prescribed antibiotic in the Faroe Islands, Iceland and Denmark (Adriaenssens et al., 2011a). The total penicillin sales (J01C) in the Faroe Islands were 11.5 DID in 2005, which decreased slightly to 10.4 DID in 2011. The sales in the Faroe Islands were comparable to Denmark, which increased from 10.0 DID in 2005 to 11.9 DID in 2011 and to Iceland, where the sales were 10.3 DID in 2005 and 12.1 DID in 2011. However, there was a difference within the J01C subgroups (Figure 6). The mean sale in Iceland of broad-spectrum penicillin (J01CA) was 4.1 DID and the mean sale of combinations with beta-lactamase inhibitors (J01CR) was 3.8 DID. This is higher than the mean sales in the Faroe Islands (2.9 DID) and Denmark (3.6 DID), for broad-spectrum penicillin (J01CA). Furthermore, the mean sale for combinations with beta-lactamase inhibitors (J01CR) was much lower 0.2 DID and 0.5 DID in the Faroe Islands and in Denmark, respectively (Figure 6). The explanation for the high use of broad-spectrum and combinations with beta-lactamase penicillins in Iceland is probably due to the circulation of multi-resistant pneumococci and a switch from normal dosing to high doses of amoxicillin in children (Hjálmarsdóttir & Kristinsson, 2014). Broad-spectrum penicillins J01CA deceased from 3.3 DID in 2009 to 2.2 DID in 2011 in the Faroe Islands. The probable explanation for the decrease in the use of broad-spectrum penicillin's in 2011 is that pivmecillinam (Selexid) and pivampicillin (Pondocillin) were

taken off the market in the Faroe Islands due to the rare genetic disease, PCD (Mørkøre et al., 2011; Rasmussen et al., 2012).

The narrow-spectrum penicillin J01CE was the most commonly used penicillin in the Faroe Islands and Denmark, with a similar use to that of in Norway and Sweden in 2009 (Versporten et al., 2011).

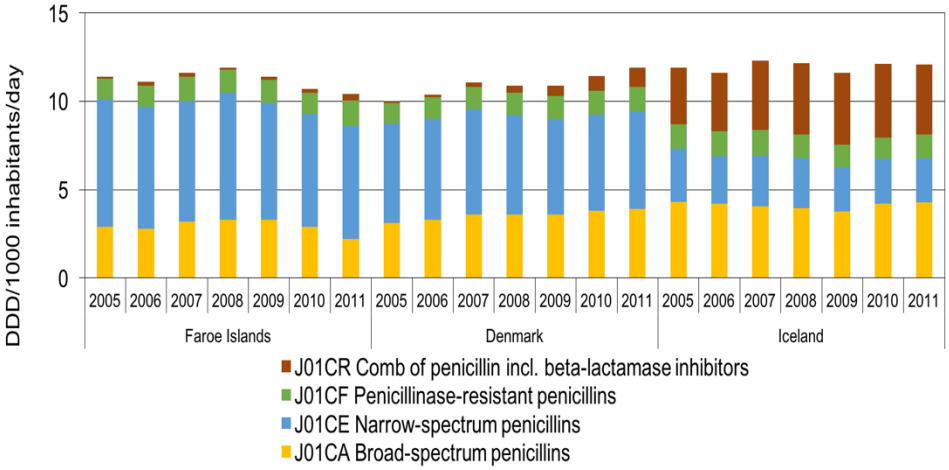

Figure 6. Sales of penicillin sub-groups (ATC J01CA, CE, CF and CR), presented in defined daily doses/1000 inhabitants/day (DID) in the Faroe Islands, Denmark and Iceland, 2005 -2011.

Macrolides J01FA

The mean sale of the macrolides subgroup (J01FA) was highest in Denmark (2.4 DID), followed by the Faroe Islands (1.9 DID) and lowest in Iceland (1.6 DID). There was a difference in the sub-groups between the countries. The Faroe Islands did mainly use erythromycin (53%), and Denmark used roxithromycin (46%) and

Iceland used azithromycin (54%) (Figure 7). DID is the assumed averaged maintenance dose per day for a drug that is mainly indicated for adults. Therefore, the antibacterial use is underestimated in children, and we determined it was necessary to analyse the prescriptions with respect to macrolides. In the Faroe Island the short-acting erythromycin was prescribed mainly to children in the age group 0-4 years and the long-acting azithromycin was prescribed to teenagers and young adults (Figures 8 & 9). A similar pattern to the reports from Denmark was found. In Iceland, the azithromycin was mainly prescribed to children in the age group 0-4 years. The pattern of azithromycin prescription in the three countries most likely reflects its main indicated utility, i.e. for chlamydia in the Faroe Islands, Denmark and Iceland, and additionally otitis media for children and respiratory tract infections for the elderly in Iceland. Clarithromycin has been recommended as the standard macrolide and for most European countries clarithromycin is the most used sub-group within macrolides, except for the Faroe Islands, Denmark and Iceland (Adriaenssens et al., 2011b).

It is unknown why the intermediate-acting roxithromycin is not used on the Faroe Islands to the extend that it is in Denmark, and in many other European countries (Adriaenssens et al., 2011b). Denmark has been using roxithromycin for several years, because it is associated with fewer side-effects than the short-acting erythromycin and it is available at a lower cost than other macrolides. The erythromycin sales in the Faroe Islands is

comparable to the erythromycin use in UK and Norway (Adriaenssens et al., 2011b). The level of erythromycin use in the age group 0-4 years in the Faroe Islands is worrisome, and is an indication that erythromycin is prescribed inappropriately here in the Faroe Islands.

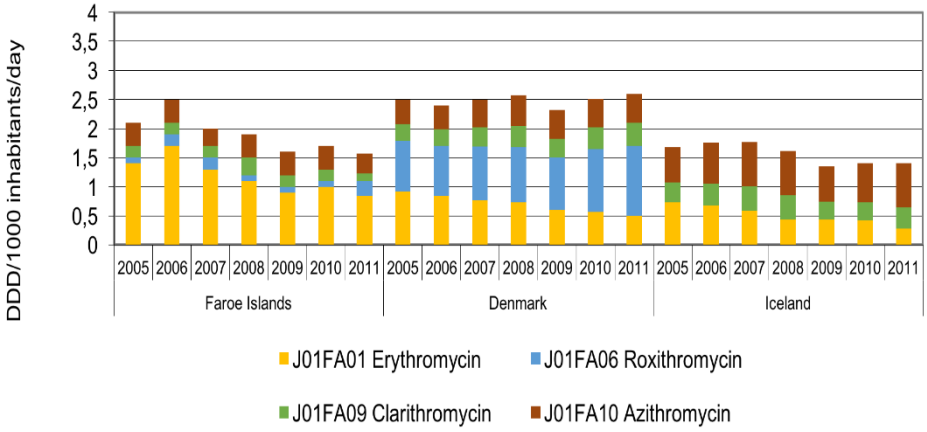

Figure 7. Sale of macrolides sub-groups (ATC J01FA01, A06, A09 and A010), presented in defined daily doses/1000 inhabitants/day (DID) in the Faroe Islands, Iceland and Denmark, 2005-2011.

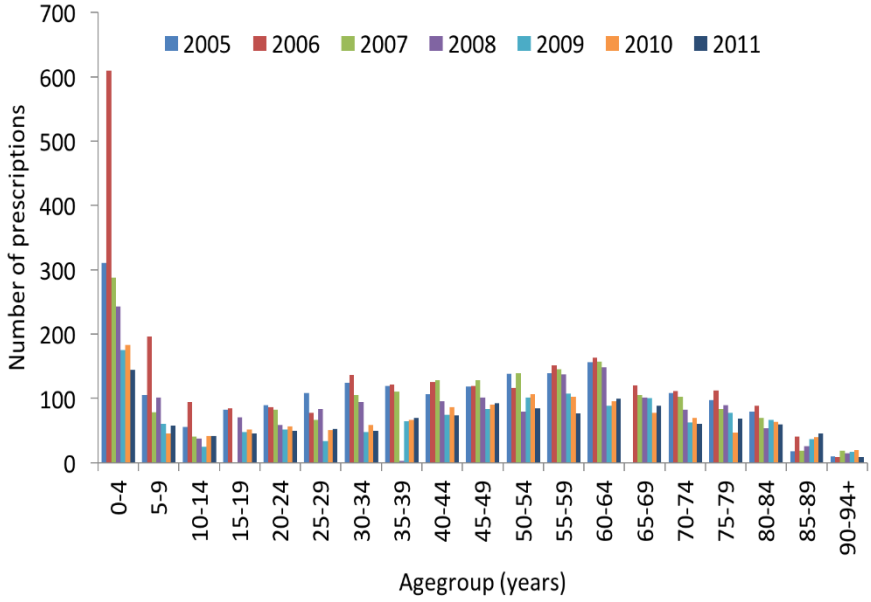

Figure 8. Prescriptions of erythromycin J01FA01 according to age groups, presented in number of prescriptions in the Faroe Islands, 2005-2011.

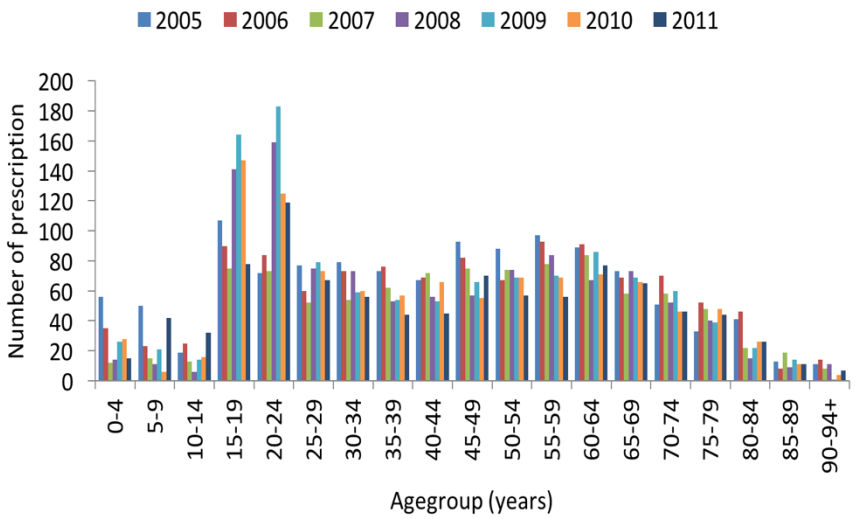

Figure 9. Prescriptions of azithromycin J01FA10 according to age groups, presented in number of prescriptions in the Faroe Islands, 2005-2011.

Sulphonamides and trimethoprim J01E

The sulphonamides and trimethoprim J01E sub-group accounted for a relatively minor part of the total J01 antibacterial sales in the three countries. The mean sale of sulphonamides and trimethoprim's was 1.2 DID, 0.9 DID and 1.4 DID in the Faroe Islands, Denmark and Iceland, respectively.

Trimethoprim/sulfamethoxazole (SXT) J01EE01 was first used in the Faroe Islands in 2010. Prior to 2010 the mean use of trimethoprim J01EA01 was 0.7 DID and of sulfamethizol J01EB02 was 0.5 DID. In Denmark, SXT is considered to have too many

side effects and is not recommended for long term-use (Engberg et al., 2017). Therefore, the two subgroups trimethoprim and sulfamethizol are used in Denmark. The mean use in Denmark of trimethoprim J01EA01 was 0.5 DID and sulfamethizol J01EB02 was 0.3 DID. The Faroe Islands and Denmark are the only countries using sulfamethizole in Europe (Coenen et al., 2011). The mean use in this group in Iceland was trimethoprim J01EA01 (0.7 DID) and SXT, J01EE01 (0.9 DID).

Cephalosporin's J01D

First generation cephalosporins were not sold in the Faroe Islands in the period 2005 to 2011, and were sold only in low amounts in Denmark. The average use of cephalosporin's J01D was 0.4 DID, 0.4 DID and 0.6 DID in the Faroe Islands, Denmark and Iceland, respectively. The use of the first-generation cephalosporin, cephalexin was the primary cephalosporin used in Iceland (0.3 DID) in the period 2005-2011. The second-generation cephalosporin, cefuroxime was sold in the amount of 0.3 DID, 0.3 DID and 0.2 DID in the Faroe Islands, Denmark and Iceland, respectively. The use of third-generation cephalosporin's was minimal in all three countries, as seen in other European countries, with the exception of southern Europe (Adriaenssens et al., 2011a). This could be due to the fact that cephalosporins are mainly reserved for hospital use.

Quinolones J01M

The use of fluoroquinolones increased in all three countries from 2005 to 2011, in Faroe Islands from 0.3 to 0.6 DID, in Denmark from 0.4 to 0.7 DID and in Iceland 0.7 to 1.1 DID. The main fluoroquinolone used was ciprofloxacin with a mean use of 0.4 DID, 0.7 DID and 0.9 DID in the Faroe Islands, Denmark and Iceland, respectively. Even if the use increased, the low use of ciprofloxacin suggests that the use is more appropriate in the Faroe Islands, Iceland and Denmark, compared to an inappropriate usage in other European countries (Naber, 2000).

Other antibiotics J01X and lincosamids J01FF and aminoglycosides J01GB

Nitrofuran J01XE was the main antibiotic used in the group comprised of other antibiotics not previously mentioned, with an average use of 0.8 DID, 0.5 DID and 0.5 DID in the Faroe Islands, Denmark and Iceland, respectively. A low use of lincosamides and aminoglycosides was recorded in all three countries with 0.06 DID, 0.13 DID and 0.0 DID of lincosamides and 0.1 DID, 0.3 DID and 0.1 DID of aminoglycosides in the Faroe Islands, Iceland and Denmark, respectively.

4.2 Antibacterial resistance in GAS from healthy carriers and tonsillitis patients (paper II).

Antibacterial resistance in GAS from healthy carriers and tonsillitis patients was described in paper II and associated with antibacterial sales in the Faroe Islands.

4.2.1 Summary

Similar to the antimicrobial use in the Faroe Islands (Paper I), information about GAS resistance prevalence in this community is also lacking. In Paper I, a difference in macrolide and tetracycline use between the Faroe Islands and Iceland was revealed. This previous study also suggested a potentially higher level of erythromycin resistance among GAS isolates in the Faroe Islands, especially among the healthy children, because erythromycin was prescribed inappropriately to children. The objective of Paper II was to examine the prevalence of antibacterial resistance in GAS isolates from the Faroe Islands, and compare the findings with Iceland, and, finally to correlate those findings to antibacterial sales (from Paper I), especially with respect to erythromycin and tetracycline.

Novel data on the GAS resistance in the Faroe Islands was generated by a comprehensive survey involving 12 of the country's 24 GP's. The resistance rate to erythromycin in the Faroe Islands was only 6% in 2009/2010, and thus lower than expected. The resistance to this drug was also generally low in

Iceland (< 10 %, Figure 8), but there was a peak in erythromycin resistance observed in 2008 (44%). In a similar fashion, the tetracycline resistance in the Faroese patients declined significantly from 2009 (37%) to 2010 (10%), (p = 0.006). Although antimicrobial use likely influences the prevalence of erythromycin and tetracycline resistance in the Faroe Islands and Iceland, this is unlikely to explain the observed sudden changes.

We thus suggest that the resistance peaks are caused by intermittent introductions of novel resistant clones from abroad. A significant difference in tetracycline resistance between age groups was also observed in the Faroe Islands. A link to tetracycline consumption is, however, unlikely as one of the highest resistance was in the youngest age group which hardly used any tetracycline. A more likely explanation is that tetracycline-resistant isolates are being transmitted between family members, and that one or a limited number of clones may be circulating in this small community at any one time. Paper II proposed a follow-up study on the clonality of GAS in the Faroe Islands and, interestingly, this work is presently being picked up by Prof. James Musser's group, Houston Methodist Hospital, Houston, Texas. Isolates from this study were sent to Houston where whole genome sequencing is being performed to genetically characterize and compare these isolates with other countries in Europe and North America. From this study, it will be of special interest to see if the GAS isolates are genetically distinct due to the small population and isolated location of Faroe Islands.

4.2.2 Demographics

In this study 169 GAS isolates were included, from two studies conducted from January to March in 2009-2010. In study I, GAS isolates came from patients, where oropharyngeal swabs were collected by twelve GPs. In study II, GAS isolates were obtained from nasopharyngeal swabs collected in the pneumococcal carriage study. There were 125 isolates from oropharyngeal swabs and 44 isolates from nasopharyngeal swabs. The mean age of the GAS carriers was 4.4 years and the mean age of the patients was 25.4 years.

4.2.3 Antibacterial susceptibility to erythromycin and clindamycin

Study I (patients): From the 125 GAS isolates the non-susceptibility to erythromycin was 6% of the total samples in 2009 and 2010. In 2009, it was 5% and in 2010 it was 10% (Table 1).

Study II (carrier): From the 44 samples the resistance to erythromycin among healthy children was 2% in 2009 and 2010. The non-susceptibility to erythromycin was 3% and 0% in 2009 and 2010, respectively (Table 1).

The resistance to clindamycin was minor in 2009, but had an increase from 2009 to 2010 from 0 to 7% (n = 1) in carriers, (Table 1).

Table 1. Susceptibility testing of GAS isolates from patients and carriers in the Faroe Islands, 2009 and 2010, with *p-values* from patients (P) and carriers (C).

	2009		*2010*		*Total isolates*		*2009/2010*	*Diff. 2009-2010*	
	Patients	*Carriers*	*Patients*	*Carriers*	*Patients*	*Carriers*	*Patients/Carriers*	*P*	*C*
R/I %	*n = 95*	*n = 30*	*n = 30*	*n = 14*	*n = 125*	*n = 44*	*n = 169*	*< 0.05*	*<0.05*
Penicillin	0	0	0	0	0	0	0	NA	NA
Erythromycin	5	3	10	0	6	2	5	0,40	1,00
Clindamycin	1	0	3	7	2	2	2	0,42	0,32
Tetracycline	37	3	10	7	30	5	24	0,006	0,54

4.2.4 Antibacterial susceptibility to erythromycin in Iceland

In 2007, the resistance to erythromycin started to increase and peaked in 2008 (44%), followed by a decline in 2009 (5%). From 2009 until 2012 the resistance prevalence to erythromycin was below 10% (Figure 10).

Despite the lower use of macrolides in Iceland the resistance prevalence to erythromycin was higher in Iceland than in the Faroe Islands. The possible explanation for this could be the use of long-acting azithromycin, which is more likely to induce resistance (Baquero, 1999; Bergman et al., 2006). Studies from Finland and France and other countries have shown a connection between lowering the resistance level in macrolide and reducing the use of macrolide (D'Humières et al., 2012; Desjardins et al., 2004; Seppala et al., 1997; Seppala et al., 1993), therefore the low use of azithromycin in the Faroe Islands can possibly explain the low erythromycin resistance prevalence. However, the results

from Iceland suggest a circulation of an erythromycin-resistant clone as seen in other studies in Finland and Portugal (Kataja et al., 1998; Silva-Costa et al., 2015).

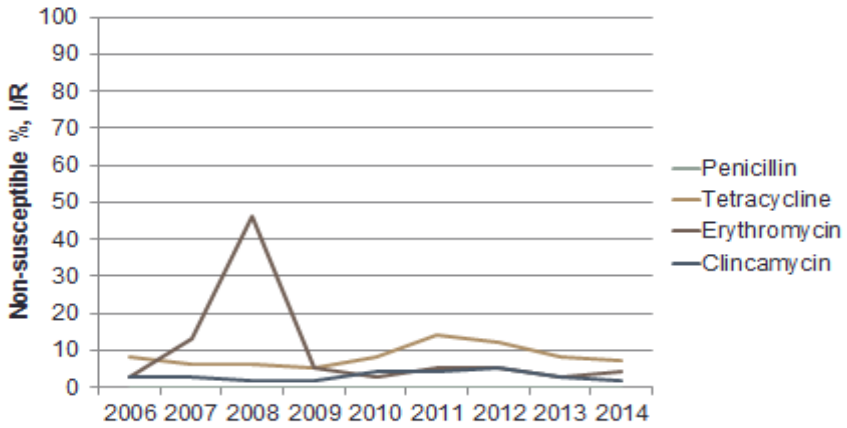

Figure 10. Resistance prevalence of GAS isolates submitted to the clinical microbiology laboratory at the Landspitali University Hospital in Reykjavik, 2006-2014.

4.2.5 Antibacterial susceptibility to tetracycline

Study I (patients): The mean percentage of isolates demonstrating tetracycline resistance among patients in 2009-2010 was 30%. In 2009 (37%) it decreased significantly (*p-value* = 0.006 < 0.05) to 10% in in 2010 (Table 1). The tetracycline resistance was significantly associated with age groups (0-15 years and 25-40 years) *p-value* = 0.03 < 0.05 (Figure 11).

There is a low use of tetracycline in the Faroe Islands (Figure 5) and no interventions have taken place to lower the tetracycline use. Mathematical models predict that, once resistance has been selected, only drastic reductions in antibiotics use can lead to reduction in resistance prevalence (Austin et al., 1999). Therefore, we can speculate that the decline in tetracycline resistance from 2009 to 2010 is partly clone related. Tetracycline resistance is significantly associated with age and the main consumers of tetracycline in the Faroe Islands are teenagers and young adults. However, the tetracycline resistance is most likely not related to tetracycline consumptions, when the resistant isolates are found among children (Figure 11). A more likely explanation could be that tetracycline resistant isolates are being transmitted from family members. In a small population, it is also possible that one or very few clones are circulating in the community at any given time.

Study II (carriers): The mean resistance to tetracycline was 5% in 2009 and 2010. It was 3% and 7% in 2009 and 2010, respectively (Table1).

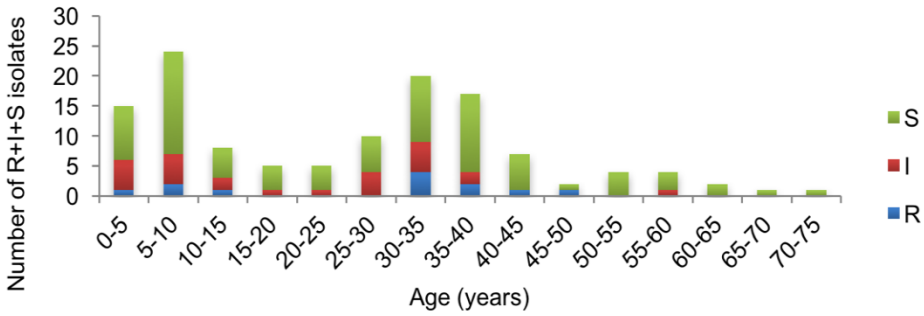

Figure 11. Number of tetracycline resistant (R), intermediate (I) and sensitive (S) GAS isolates from patients in the Faroe Islands, 2009-2010.

4.3 Antibacterial resistance in *E. coli* from community-acquired UTIs in the Faroe Islands (paper III).

Antibacterial resistance in *E. coli* from community-acquired UTIs was described in paper III and associated with antibacterial sales in the Faroe Islands, and compared with Iceland and Denmark.

4.3.1 Summary

The approach in this work follows the outline for Paper II – here with a focus on the *E. coli* bacteria. Data on the antibacterial susceptibilities of *E. coli* in the Faroe Islands were lacking before the initiation of this work. Our broad survey of urine samples from patients (2009, 2010 and 2012), involving half of the country's 24 GP's, filled the knowledge gap and provided the required data. These new data were analysed together with the antimicrobial use in the Faroe Islands (Paper I) and with similar data from Iceland and Denmark. Non-susceptibility to at least 1 of the 14 antibacterial drugs investigated was found in 54% of the *E. coli* isolates and was most common to ampicillin (46%), followed by sulfamethoxazole (39%), trimethoprim (27%), trimethoprim/sulfamethoxazole (27%), and < 10% to the remaining 10 antibiotics. The resistance prevalence did not change significantly with time. We found significant linear relationships between antibacterial mean sales and antibacterial resistances. In addition, we have developed a novel logistic modelling approach, which facilitates realistic predictions of the future developments in resistance with increased antibiotic sales.

The resistance increases induced by the different types of antibiotic are categorized into two groups – ‘steep’ and ‘gradual’. This knowledge can potentially be used to predict and control the future increase in *E. coli* resistance with antibacterial sales – e.g. prescriptions of drugs within the ‘steep’ group should be given in moderation, since these can result in a more rapid increase in resistances. The high resistance level of sulphonamide, combined with the fact that this drug is still considered the first-choice treatment for uncomplicated UTIs in the Faroe Islands, is worrisome. Furthermore, the association between antibacterial resistance and antibacterial use in the Faroe Islands, Iceland and Denmark justifies a re-evaluation of antibacterial policies against UTIs treatment in the Faroe Islands.

4.3.2 Demographics

During the study period 2009-2012, there were 24 GP’s in the Faroe Islands, and of twelve of them participated in this study.

In this study 210 *E. coli* isolates were included, from the period 2009 and 2010 (January to March). From June to December 2010 and April to December 2012 additional *E. coli* isolates were included in this study. These isolates were collected at GP clinic and processed at the department of clinical microbiology at the National Hospital in the Faroe Islands. In the sampling period 2009, there were 27 *E. coli* isolates, in 2010 there were 63 and in 2012 there were 120 *E. coli* isolates. Due to the small number of isolates in 2009 and 2010, the years were combined. The *E. coli*

isolates were mainly from women, 178 (85%) and 32 (15%) from men.

4.3.3 Antibacterial susceptibility

UTI's are treated empirically in the Faroe Islands without culture or susceptibility data, generally with sulfamethoxazole as the first line drug. Susceptibility testing was done for fourteen antibiotics. The resistance prevalence in all isolates was highest for the following four antibiotics, ampicillin (46%), sulphonamide (39%), trimethoprim (27%) and sulphoamethoxazole/trimethoprim (27%). Co-amoxiclav and nalidixic acid was below 10% and the remaining eight antibiotics was below 5% (Table 2).

Table 2. Susceptibility testing of *E. coli* isolates from community UTIs in the Faroe Islands, 2009, 2010 and 2012.

	2009/2010		*2012*		*Total isolates*			
							95% CI	
	n = 90	*R %*	*n = 120*	*R %*	*n = 210*	*R %*	*Lower*	*Upper*
Ampicillin	39	43	57	48	96	46	39	53
Cefoxitin	2	2.2	2	1.7	4	1.9	0.5	4.8
Trimethoprim	23	26	34	28	57	27	21	34
Trim/Sulpha	23	26	33	28	56	27	21	33
Nitrofurantoin	3	3.3	6	5.0	9	4.3	2.0	8.0
Mecillinam	1	1.1	0	0	1	0.5	0	2.0
Cefpodoxime	2	2.2	4	3.3	6	2.9	1.1	6.1
Co-amoxiclav	5	5.6	8	6.7	13	6.2	3.3	10
Cefuroxime	1	1.1	2	1.7	3	1.4	0.3	4.1
Ceftizoxime	0	0	0	0	0	0	0	1.7
Nalidixic acid	4	4.4	7	5.8	11	5.2	2.6	9.2
Gentamicin	2	2.2	2	1.7	4	1.9	0.5	4.8
Ciprofloxacin	3	3.3	3	2.5	6	2.9	1.1	6.1
Sulphonamide	33	37	49	41	82	39	32	46

4.3.4 Antibacterial susceptibility correlated with antibacterial sales in the Faroe Islands

As a result of establishing an antibiogram, we found a significant association between antibacterial resistance among *E. coli* and antibacterial sales in the Faroe Islands which is consistent with other studies (Goossens et al., 2005). Two different methods were applied. First, a trend test with data from the Faroe Islands was performed without using any limitations or excluding data points. Resistance prevalence (R+I) of seven antibiotics (gentamicin, co-amoxiclav, ciprofloxacin, nitrofurantoin,

mecillinam, sulphonamide and ampicillin were significantly correlated with the sale of aminoglycosides, amoxicillin and enzyme, quinolones, nitrofuran, pivmecillinam, SXT and penicillin using *prop.trend.test* in resistance prevalence (R+I) with antibacterial mean sales (*p-value* = $2.2 \cdot 10^{-16}$).

Second, a revised quasi-binomial logistic regression of five antibiotic resistance prevalence in the Faroe Islands versus mean sales in 2008-2011 was performed. By excluding mecillinam and co-amoxiclav acid the overdispersion went from ≈ 31 to ≈ 1, hence five antibiotics were tested instead of seven, as was done in trend test.

There was a significant association between the mean of antibiotic resistance (5 drugs) in 2009-2012 and the mean antibacterial sale 2008-2011 in the Faroe Islands *p-value* = 0.0007 (Figure 12).

In the Faroe Islands susceptibility testing is not routinely performed before treatment of UTIs in the primary health care sector, and UTIs are treated with short courses of empirical antibiotics, with sulphonamides still considered as one of the first-choice treatments for uncomplicated UTIs. Therefore the high resistance prevalence in sulphonamides is worrisome, especially when comparing with Denmark, Sweden and Norway where the resistance prevalences of SXT, trimethoprim and sulphonamides were much lower (Kahlmeter, 2003). In Denmark sulphonamide resistance prevalence is decreasing from 2009 to 2012 (Table 3 in paper III). The resistance prevalence was also high to

trimethoprim and SXT. The high resistance prevalence in SXT is probably due to the constant use of sulphonamides and trimethoprim since SXT has not been sold in large amounts and usage was first recorded in 2010 in the Faroe Islands.

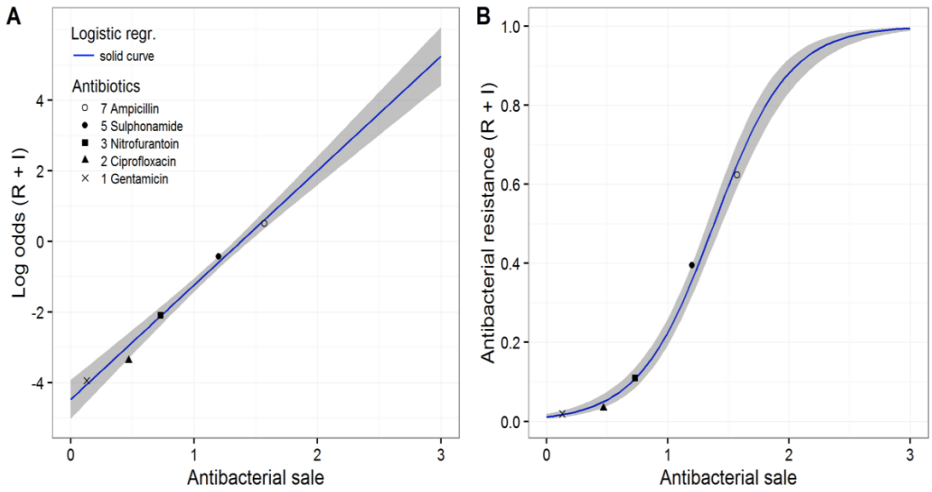

Figure 12. Logistic regression of five antibiotic resistance (R+I) proportions in the Faroe Islands versus mean sales in 2008-2011 DID. The plot A in on the log odds scale and the plot B is on the probability scale. We found $ß_1 = 3.24$ for slope (Std. Error = 0.226, *p-value* = 0.0007).

4.3.5 Antibacterial susceptibility correlated with antibacterial sales in the Faroe Islands, Iceland and Denmark

We found a significant association between antibacterial resistance and antibacterial sales in the Faroe Islands, Iceland and Denmark by using two different statistics methods, Pearson's product-moment with a strong correlation (cor. = 0.78, *p-value* = 0.0001) and Spearman's rank with a strong correlation (rho = 0.68, *p-value* = 0.002) (see Tables 2 and 3 in paper III).

By modelling antibacterial resistance versus antibacterial sales for the Faroe Islands, Iceland and Denmark with two different antibiograms, one with steep slope and one with gradual slope, our results suggest that ciprofloxacin, nitrofurantoin and sulphonamide follow a steep increase with antibacterial sales (Figure 13). Ampicillin, co-amoxiclav, gentamicin and mecillinam, on the other hand follow a more gradual increase with antibacterial sales (Figure 14). Our result proposes both gradual and steep development in antibacterial resistance, which can possibly be used to predict and control the future increase in *E. coli* resistance with antibacterial sales. However, with this small dataset we must be cautious with extending the interpretation of these data for other countries; instead viewing it as a suggested effect observed locally in this small subset of Nordic countries. As seen in other studies, we are demonstrating a strong increase in resistance with sales in the model and the high slope could have a large impact on resistance level (Bell et al., 2014). According to this model, we can hypothesize that by staying under the

antibiotic sale level in sub-groups of 0.5 DID the resistance rate would be low and between 0.5 DID to 1.0 DID the resistance would be below 20% (Figure 13). There was a concern for a higher use of ciprofloxacin when pivmecillinam and pivampicillin was withdrawn from the Faroese market in 2011 to avoid risks related with PCD (Mørkøre et al., 2011; Rasmussen et al., 2012). Ciprofloxacin is placed in the steep increasing group in this study, and therefore higher sales would justify a concern since fluoroquinolones are not recommended for uncomplicated UTIs due to the risk of increased resistance (Naber, 2000). The resistance to ciprofloxacin did not increase in the Faroe Islands nor did the sales, however the sale of sulphonamide did increase in 2011, probably as a side-effect of the withdrawal of pivmecillinam and pivampicillin.

Most Nordic countries use mecillinam as the first choice treatment of uncomplicated UTIs (Naber, 2000). The resistance prevalence is low in the Faroe Islands and mecillinam is placed in the gradual increasing resistance group (Figure 14), which is comparable to other Nordic countries such as Norway and Sweden (Kahlmeter, 2003). This low resistance prevalence in the Faroe Islands is probably because of the withdrawal of pivmecillinam from the Faroese market and the decrease in sale of pivmecillinam from 2010 to 2011, directly preceding the withdrawal.

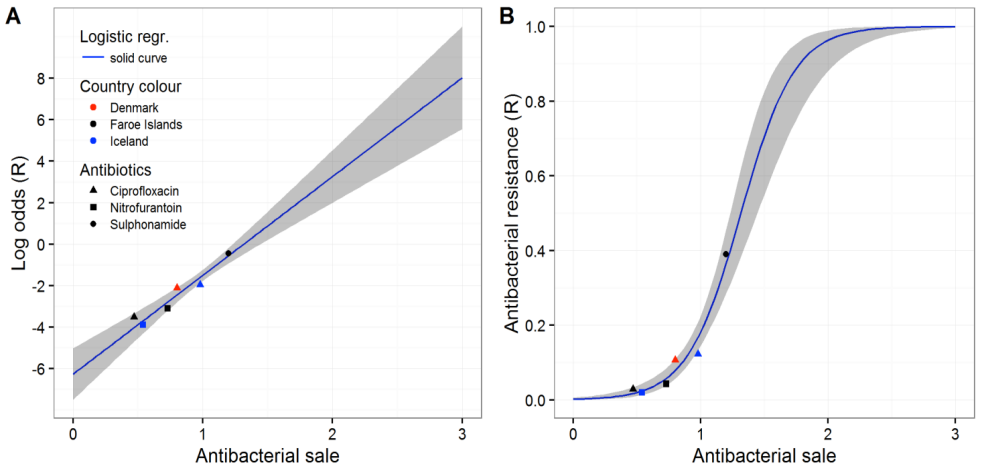

Figure 13. Logistic regression of six steep increasing antibiotic resistance prevalence (R) for three antibiotic (ciprofloxacin, nitrofurantoin and sulphonamide) versus mean sales 2008-2011. Overdispersion is moderate (≈ 2), and we find $ß_1 = 4.77$ (Std. Error = 0.624, *p-value* = 0.002).

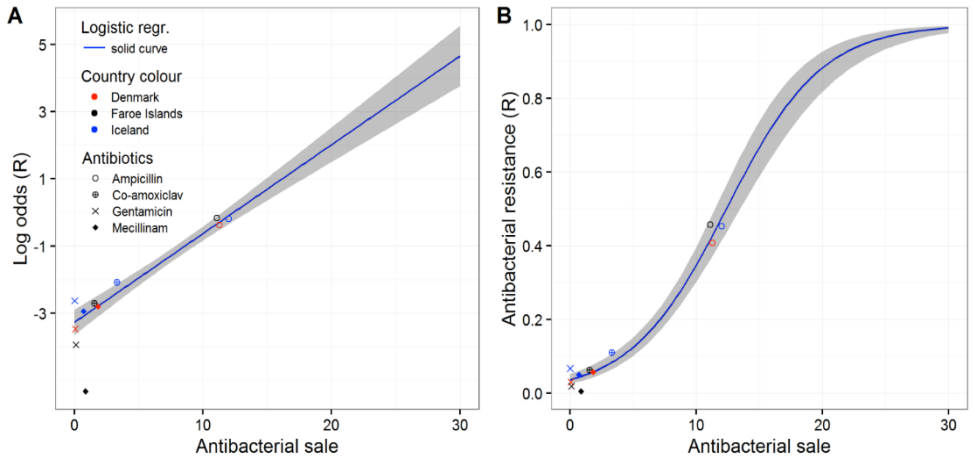

Figure 14. Logistic regression of 11 gradual increasing antibiotic resistance prevalence (R) for four antibiotic (ampicillin, co-amoxiclav, gentamicin and mecillinam) versus mean sales 2008-2011. Overdispersion is moderate (≈ 2), and we find $ß_1 = 40.26$ (Std. Error = 0.020, *p-value* = $4 \cdot 10^{-7}$).

4.4 Pneumococcal carriage, antibacterial resistance and serotype prevalence (paper IV).

Pneumococcal carriage, antibacterial resistance, and serotype prevalence in healthy pre-vaccinated children and healthy children enrolled in the vaccine program aged 0-7 years attending DCCs is described in paper IV. Results obtained from invasive isolates in the Faroe Islands is also described.

4.4.1 Summary

This final paper is also an attempt to amend the limited data available on bacteria resistance in the Faroe Islands (like Papers II-III). Prior to this study, we had no knowledge on carriage prevalence, antibacterial resistances and serotype prevalence after the introduction of PCV7/13 in this country. The main objective was to describe and compare carriage, antibacterial resistance and serotype prevalence of pneumococci in healthy children aged 0 to 7 years, in invasive pneumococci from 1974 to 2016, and to compare the results with similar studies from Iceland and Denmark. Our main results are that pneumococcal carriage prevalence in children attending DCCs is low and antibacterial resistance in pneumococci is presently extremely rare in the Faroe Island.

The prevalence of PNSPs was low compared to Iceland and Denmark, likely because of the conservative use of antibiotics in the Faroese community. The established vaccine program in the Faroese community in 2008 seems to have reduced

incidence of PCV-7/13 serotypes in IPD. Furthermore, there is an indication of serotype shift in pneumococcal carriage. One can speculate whether this serotype shift has taken place after the introduction of PCV-7 and the serotypes among serotype PCV-13 is delayed as seen in Norway (Steens et al., 2015). Follow-up studies regarding antibacterial susceptibility, use and serotype data are, however, required in order to confirm this suggestion.

4.4.2 Demographics

In this study 607 pneumococcal isolates were included from a cohort study conducted in January to March in 2009-2011. The study covered 11% of children aged 0-7 years in the Faroe Islands. 60 invasive isolates were also included. Information about age, sex, susceptibility testing, serotype and origin of the pneumococcal isolates (blood, etc.) from 1974 to 2016 was obtained from the Statens Serum Institut (SSI, Copenhagen, Denmark).

4.4.3 Pneumococcal carriage

The mean pneumococcal carriage prevalence in the years 2009, 2010 and 2011 was 45%. With a significant decrease from 2009 (50%) to 40% and 42% in 2010 and 2011, respectively ($X^2 = 6.03$ with a critical region < 5.991) (Table 3). The carriage prevalence was significant higher in children younger than three years old *(p*

value = 0.001) and the mean age was significantly lower in 2009 (*p value* = 0.011) (Figure 15). The pneumococcal carriage is lower in the Faroe Islands than in Denmark and Iceland (Hjálmarsdóttir et al., 2015; Slotved et al., 2016), however the association with low age is comparable to neighbouring countries, such as Iceland and Norway (Steens et al., 2015; Tomasson et al., 2005).

Antibiotic use and OM are often associated with pneumococcal carriage and antibiotics may for a period reduce carriage of pneumococci in general, but may increase the rate of carriage of non-susceptible pneumococci (Arason et al., 1996; Zenni et al., 1995). This was not the case in this study, antibiotic use and OM was not associated with carriage. In addition, antibiotic use and OM was not more common among children carrying pneumococci, nor was having siblings under the age of six years of age found to be more common among pneumococcal carriers compared to non-carriers in 2009, 2010 and 2011 (Table 7 in Appendix I). Pneumococcal carriers did not use antibiotics more often than non-pneumococcal carriers. However, pneumococcal carriers used antibiotics more often in 2009 (*n* = 43) than pneumococcal carriers in 2010 (*n* = 10) and 2011 (*n* = 24) (*p value* = 0.017). This finding could suggest that there is a reduced antibacterial use after the introduction of PCV-7 and PCV-13.

There was variation in pneumococcal carriage prevalence between the DCCs, which may partially account for the difference in carriage between the years (Figure 16).

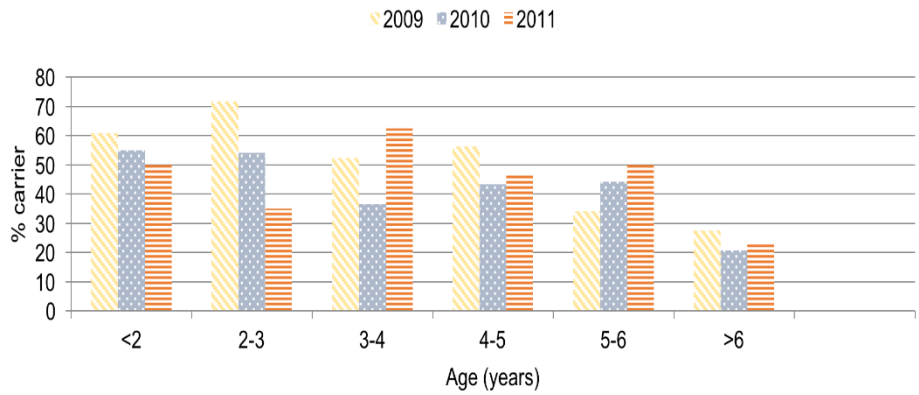

Figure 15. Pneumococcal carriage prevalence versus age from healthy children attending DCCs in the Faroe Islands in 2009, 2010 and 2011.

Table 3. Characteristics of pneumococcal carriers (*n* = 271) and non-carrier (*n* = *336*) attending DCC'S in the Faroe Islands in 2009, 2010 and 2011. X^2 = Critical region with df = 2 is <5.991, $\propto$ 0,05

		Year		*Signifiance*
Parameter	*2009*	*2010*	*2011*	
Children sampled (n)	265	225	117	
Total isolates (n)	133	89	49	
Carriage (%)	50	40	42	$X^2 = 6.03$
Mean age, carrier (yr)	3.49	4.24	4.07	$p = 0.0002$
Mean age, non-carrier (yr)	4.35	4.65	4.34	

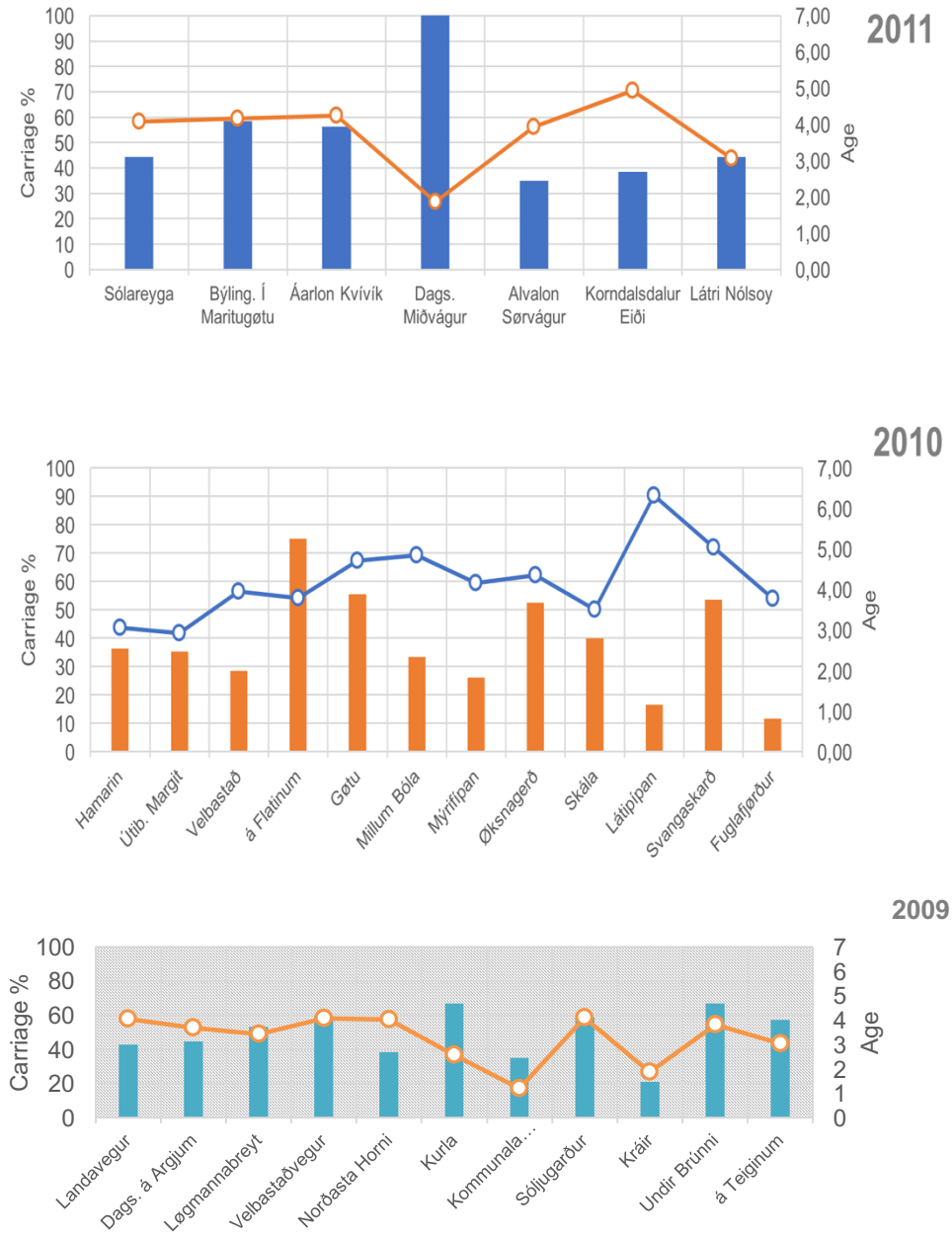

Figure 16. Pneumococcal carriage prevalence versus age from healthy children attending the 30 DCCs in the Faroe Islands in 2009, 2010 and 2011.

4.4.4 Antibacterial susceptibility

Reduced susceptibility to one or more antimicrobial agent was found in 15 (11.3%), 8 (9.0%) and 7 (14.3%) of the pneumococcal isolates in 2009, 2010 and 2011, respectively.

Erythromycin resistance was 1.5% (*n* = 2), 1.1% (*n* = 1) and 1.1% (*n* = 1), in 2009, 2010 and 2011, respectively (Table 4). These four isolates belonged to three different serotypes (19F (*n* = 2) in 2009, 6C in 2010 and 11 in 2011. The erythromycin resistant isolates of serotype 6C were also non-susceptible to penicillin. Resistance among the pneumococcal isolates was highest to SXT in 2009 and 2010 while the highest non-susceptibility was to tetracycline in 2011 (Table 4).

The prevalence of PNSP was low in the Faroe Islands compared to Iceland (Arason et al., 2006; Erlendsdottir et al., 2001; Hjálmarsdóttir & Kristinsson, 2014; Sigurdsson et al., 2017). Five PNSPs were found, all five were intermediate to penicillin. Three in 2009 and two in 2010 (Table 5). The five PNSPs were of the following serotypes NT, 19F (*n* = 2) in 2009 and 6C and 23 (not serotype F) in 2010 (Table 5). Antibacterial susceptibility among the pneumococcal isolates is not comparable to Iceland, but is comparable to other Nordic countries, such as Denmark, Sweden and Norway (Hogberg et al., 2007; Nielsen et al., 2004; Steens et al., 2015; Vilhelmsson & Kristinsson, 2007). The low level of PNSP and antibacterial resistance can be explained in several ways; where the conservative use of antibacterials in the Faroese community is

one likely factor (Figure 4). Another probable explanation could be that the macrolide of choice is short-acting erythromycin, whereas long-acting azithromycin is often the choice in Iceland (Figure 7), even though it is not recommended. Azithromycin, may have been a contributing factor to the circulation and increase of 19F clone, which is often associated with PNSP among pneumococcal carriers (Hjálmarsdóttir & Kristinsson, 2014). The rural lifestyle in the Faroe Islands could contribute to the low antibacterial resistance, since transmission of resistant bacteria in low-density populations, such as in our study, is less frequent than in dense urban populations (Sombrero et al., 2008). Finally – in small setting such as DCCs and the Faroese community as a whole, herd protection may emerge rapidly.

Table 4. Susceptibility testing of pneumococcal isolates from healthy children attending DCCs in the Faroe Islands in 2009, 2010 and 2011.

	2009		*2010*		*2011*		*2009-2011*	*Penicillin G MIC (mg/L)*
	n = 133		*n = 89*		*n = 49*		*n = 271*	
	R/I (n)	*R/I %*	*R/I (n)*	*R/I %*	*R/I (n)*	*R/I %*	*average R/I (%)*	
Penicillins								
Oxacillin	8	6.0	6	6.7	1	1.1	5.5	
Penicillin G	3	2.3	2	2.2	0	0	1.7	0.125 to 0.25
Erythromycin	2	1.5	1	1.1	1	1.1	1.1	
Chloramphenicol	0	0	0	0	0	0	0	
Clindamycin	2	1.5	1	1.1	0	0	1.0	
Tetracycline	3	2.3	1	1.1	4	4.5	1.8	
Sulphonamides/trimethoprim	5	3.8	3	3.4	2	2.2	2.9	

Table 5. Serotypes of penicillin none susceptible pneumococci (PNSP) from healthy children attending DCCs in the Faroe Islands in 2009, 2010 and 2011.

						Serotypes of PNSP			
Year	*No. of children*	*No. of PNCa*	*(%) carriage*	*No. of PNSP*	*(%) PNSP*	*6C*	*23 not F*	*NT*	*19F*
2009	265	133	50	3	2.3			1	2
2010	225	89	40	2	2.2	1	1		
2011	117	49	42	0	0				
Total	607	271	44.6						

4.4.5 Vaccination and serotype prevalence

Among the 271 isolates, 27 different serotypes were identified. The most frequent serotypes were 6B, 6A, 6C and 19F in 2009, 3 and 6C in 2010 and 11 and 6C in 2011 (Figure 17). PCV-7 serotypes accounted for in numbers 58, 29 and 5 and PCV-13 for 32, 23 and 3 of all carried isolates in the years 2009, 2010 and 2011, respectively.

In 2008 Faroese children were vaccinated with PCV-7 and in spring 2010 PCV-7 was replaced with PCV-13. In 2010, twenty-five of the children in the study (28%) were PCV-7/13 vaccinated and nine (10%) of the twenty-five children were pneumococcal carriers and in 2011 thirty-two children (65%) were vaccinated and eighteen (37%) of the thirty-two children were carriers. There was no statistical difference in vaccination status in carriers and non-carriers in 2010 and in 2011 (Table 7 in Appendix I). In 2010, four fully vaccinated children carried vaccine serotypes, two with serotype 6B and two with serotype 19F and one child vaccinated with 2 doses carried serotype 19A. Nine children were carriers of

serotype 3 in 2010, none of them were vaccinated. In 2011 one child vaccinated with one dose carried serotype 23F and one child fully vaccinated carried serotype 3, and twelve children were carriers of serotype 11 of which four of them were fully vaccinated. Among the healthy children our results suggest a serotype shift, 6B and 6A, both PCV serotypes were main the serotypes in 2009 and 6B is not found in 2011 and 6C and 11 none-vaccine serotype (NVT) are dominant in 2011 (Figure 17).

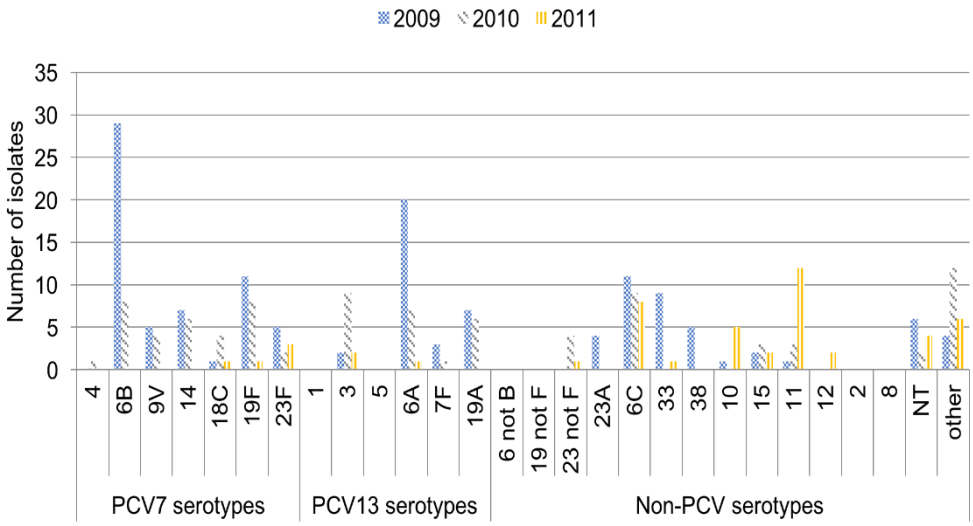

Figure 17. *Streptococcus pneumoniae* serotypes from healthy children attending DCCs in the Faroe Islands in 2009, 2010 and 2011.

4.4.6 Susceptibility and serotypes of invasive isolates

Of the 60 invasive isolates, only one PNSP was recorded. This isolate was of serotype 23B (penicillin MIC = 0.25) found in 2016. This finding is lower than in Iceland (Hjálmarsdóttir & Kristinsson, 2014), however, caution must be taken when comparing PNSP prevalence with other countries. It can be speculated that there were additional invasive pneumococci in the Faroese community, since there was no established coherent collection program of invasive pneumococci in the Faroe Islands before 2007. Three isolates were recorded as erythromycin resistant, one of serotype 15A in 2002, which also was recorded as multi-resistant. Interestingly, two erythromycin resistant isolates of serotype 14 were found in 2005 and 2006, while other studies have shown that serotype 14 is not considered to be of high invasive potential (Brueggemann et al., 2004). Some studies have correlated age to serotype 14 in IPD, since a serotype with low invasive capacity may cause opportunistic infection in children and the elderly (Brueggemann et al., 2003), however the patients carrying the erythromycin resistant serotype 14 were 80 and 26 years old. The most frequent serotype among invasive isolates were 3 (*n* = 8) and 7F (*n* = 7) (Table 6) and were also the most frequent serotypes in patients older than 7 years of age. Serotype 7F is considered to have a high invasive potential (Brueggemann et al., 2004), and therefore it is not surprisingly one of the dominant serotype among IPD.

Nine cases of serotype 3 were recorded in 2005, 2010, 2011, 2013, 2014 and 2016, and none in children < 7 years of age. This is comparable to Denmark where the vaccination program probably has not lead to a reduction of incidence of IPD caused by serotype 3 (Slotved et al., 2016). Among the 60 IPD isolates, seven were from children. One 2-year old child had the PCV-13 serotype 7F in 2010 (Table 6). From 2012 to 2016, nine NVT among fifteen serotypes from invasive isolates were found (Figure 18).

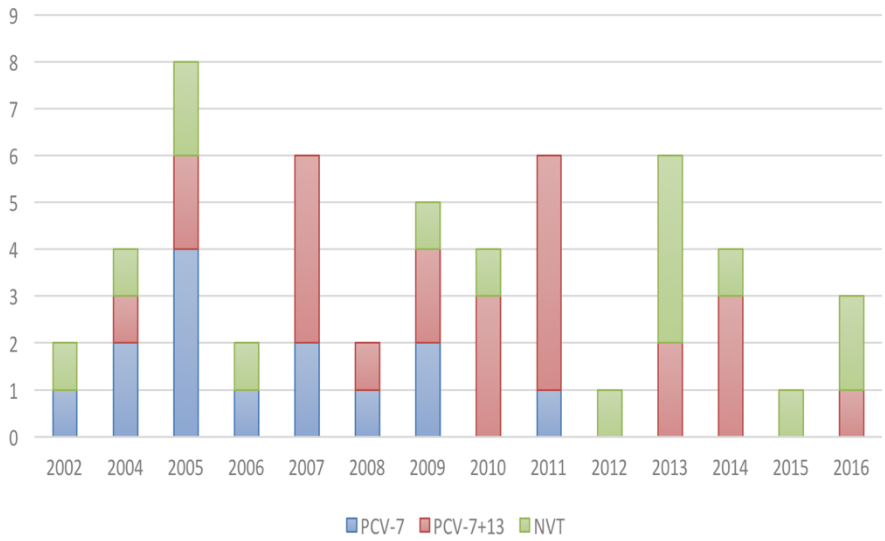

Figure 18. Serotype distribution in invasive isolates in the Faroese community, 2002-2016. PCV-7; serotypes 4, 6B, 9V, 14, 18C, 19F, 23F, PCV-13 serotypes in PCV-7 plus serotypes 1, 3, 5, 6A, 7F, 19A and NVT; other than in PCV-7 and PCV-13.

Table 6. Serotypes from invasive *Streptococcus pneumoniae* from the Faroe Islands from 1974 to 2016. Serotype marked with bold are antibacterial resistant and serotypes underlined belong to PCV7/13.

Year	Serotype
1974	34
1975	14, 7F, 8
1985	12F
1995	45
1997	-
2002	19F, **15A**
2004	33F, 14, 4, 7F
2005	4, 3, 20 (*n* = 2), 18C, **14,** 6B, 6A
2006	**14,** 22F
2007	7F, 1 (*n* = 3), 9V, 19F
2008	19F, 1
2009	7F (*n* = 2), 9V, 6B, 33F
2010	7F, 3, 12F, 19A, 11A
2011	3 (*n* = 2), 7F, 1, 23F
2012	23A
2013	22F (*n* = 2), 3 (n = 2), 35F, 15B
2014	7F, 3 (*n* = 2), 15C
2015	21
2016	3, 15C, **23B**

5 Summary and conclusions

Although the remotely located Faroe Islands could be ideal for carriage, clonal and vaccine studies, a severe lack of data has, prior to our study, limited such investigations. Our novel data collection and analysis has provided valuable knowledge on the antibacterial resistances in the three human bacterial pathogens - Group A streptococcus (GAS), *Escherichia coli* (*E.* coli) and pneumococci. By comparing this new knowledge with the neighboring countries, Iceland and Denmark, we have provided the first assessment of our community's status with regards to the global multi-resistance threat.

Compiling sales data, we found differences in total antibacterial use and in the use of individual antibacterials between the three countries. The difference was mainly caused by the higher tetracycline sale in Iceland and there was variation within the penicillin and macrolide groups in the three countries. The higher use of broad-spectrum penicillin and the combinations with beta-lactamase inhibitors in Iceland could reflect problems associated with the higher antibacterial resistance among pneumococci causing infection there.

The antibacterial use in the Faroe Islands is particularly stable, and a partial explanation for this is likely the fact that there is no antimicrobial policy and no information on antimicrobial

resistance in this country. One suspicion derived from the sales study concerns a potentially improper use of erythromycin for children in the Faroes.

There was a variation in resistance prevalence between the investigated GAS bacterial isolates in the Faroe Islands. The resistance prevalence among GAS isolates was lower than expected after the insight of improper use of erythromycin among children. We observed a sudden decrease of tetracycline resistance among GAS isolates in patients from 2009 to 2010. In similar fashion, the erythromycin resistance decreased in Iceland from 2008 to 2009. Though antimicrobial use probably influences the prevalence of erythromycin and tetracycline resistance in the Faroe Islands and Iceland, this is unlikely to explain the observed sudden changes. We thus suggest that the resistance peaks are caused by intermittent introductions of novel resistant clones from abroad. A significant difference in tetracycline resistance between age groups was also observed in the Faroes, which cannot be ascribed to tetracycline consumption, as one of the highest resistance prevalence was in the youngest age group which hardly used any tetracycline. A more probable explanation is that tetracycline-resistant isolates are being spread between family members, and that one or very few clones can be circulating in this small community at any given time.

The resistance prevalence among *E. coli* isolates was high with regard to ampicillin, trimethoprim, SXT and sulphonamides, and we found significant linear relationships between antibacterial

mean sales and antibacterial resistances. In addition, we developed a novel logistic modelling approach, which can facilitate representative predictions of the future developments in resistance with increased antibiotic sales. This model enables us to categorize the resistance increases induced by the different types of antibiotic into two groups – 'steep' and 'gradual'. This knowledge can potentially be used to predict and control the future increase in resistance with antibacterial sales – e.g. prescriptions of drugs within the 'steep' group should be given in moderation, since these can result in increased resistances.

The pneumococcal carriage prevalence in children attending day-care centres is relatively low and antibacterial resistance in pneumococci is presently very rare in the Faroe Islands. The prevalence of penicillin non-susceptible pneumococci (PNSP) was low compared to Iceland and Denmark, likely because of conservative use of antibiotics in the Faroese community. The established vaccine program in the Faroese community in 2008 seems to have reduced incidence of PCV-7/13 serotypes among invasive pneumococcal disease (IPD), and there is, furthermore, an indication of serotype shift in pneumococcal carriage.

Further monitoring is important to detect new serotypes in invasive disease and changes in pneumococcal disease prevalence.

Our study has already provided important knowledge to general practitioners regarding medical treatment, and its potential as a basis for further studies on serotype shift, vaccine impact, carriage and surveillance studies in the Faroes is apparent.

As an example, we see that the high level of resistance prevalence to sulphonamides among *E. coli* isolates is concerning, especially since they are still considered the first-choice treatment for uncomplicated urinary tract infections (UTIs) in the Faroe Islands. We suggest that the association between antibacterial resistance and antibacterial use in the Faroe Islands, Iceland and Denmark justifies a re-evaluation of antibacterial policies against UTIs treatment in the Faroe Islands. Our study on the clonality of GAS in the Faroe Islands has already prompted a follow-up study by Prof. James Musser's group, Houston Methodist Hospital, Houston, Texas. Isolates from our GAS strain collection were sent to Houston where whole genome sequencing is being performed to genetically characterize and compare these isolates with other countries in Europe and North America. From this new study, it will be of special interest to see if the GAS isolates are genetically distinct due to the small population and isolated location of Faroe Islands. Finally, as a direct result of the work completed in this thesis, a political action group has been established in the Faroe Islands to monitor and address antibacterial surveillance and stewardship.

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Original publications

Paper I

Marita Debess Magnussen, Thorolfur Gudnason, Ulrich Stab Jensen, Niels Frimodt-Møller, Karl G. Kristinsson. Antibacterial use in the Faroe Islands, Iceland and Denmark 1999-2011. *Scand J of Inf Dis,* 2014, 502-507.

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ORIGINAL ARTICLE

Antibacterial use in the Faroe Islands, Iceland, and Denmark 1999–2011

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Abstract

Background: The Faroe Islands, Iceland, and Denmark are neighbouring Nordic countries with great ethnic, cultural, and political similarities and are relatively homogeneous. Important information about prescribing practices can be obtained by comparing the antibacterial use in these countries. The objective was to describe, compare, and analyse the use of systemic antibacterial agents in these countries during the y 1999–2011. *Methods:* Data were obtained from the Faroe Islands, Iceland, and Denmark on systemic antibacterial use and expressed in defined daily dosages (DDD). Prescription data were also obtained for specific age groups. *Results:* The total antibacterial use for the y 1999–2011 varied markedly between the 3 countries, with a mean use of 21.8 DDD/1000 inhabitants/day (DID) in Iceland, 17.7 in the Faroe Islands, and 16.3 in Denmark. The total use remained fairly constant over the years in the Faroe Islands and Iceland, whereas in Denmark it increased gradually from 13.5 DID in 1999 to 19.5 DID in 2011. The higher use in Iceland can be explained by much higher consumption of tetracyclines. There was also considerable variation in the use of individual penicillins and macrolides between the countries. *Conclusions:* Despite the great ethnic and cultural similarities of these 3 countries, we found marked differences in total antibacterial use and important differences in the use of individual antibacterials.

Keywords: *Antibacterial use, Faroe Islands, Iceland, Denmark*

Introduction

Antimicrobial resistance has become one of the major threats to public health worldwide. Although the relationship between antimicrobial consumption and antimicrobial resistance may not always be direct and obvious, there is no doubt that antimicrobial consumption is one of the main reasons for the increasing antimicrobial resistance. This microbial threat was recognized at an invitational European Union (EU) meeting in Copenhagen, Denmark, in 1998, where it was recommended that the European Union and member states should collect data on the supply and consumption of antimicrobial agents and that coordinated research on antimicrobial resistance should be a high priority. This was later put into effect with the European Council "Recommendations on the prudent use of antimicrobial agents in human medicine" (2002/77/EC) [1].

The European Surveillance of Antimicrobial Consumption (ESAC) has been collecting and publishing data on the use of systemic antibiotics from 35 countries since 2001, and under the auspices of the European Centre for Disease Control (ECDC) since 2011 (ESAC-Net). The available information and the quality of the data vary and have been improving over the years, but there remain differences between the countries [2].

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No information on antimicrobial use in the Faroe Islands was published until 2009; the Faroe Islands constitute a self-governing country under the sovereignty of Denmark. Both Denmark and Iceland have collected information on antimicrobial use for a long time.

The Faroe Islands, Iceland, and Denmark are neighbouring Nordic countries with great ethnic, cultural, and political similarities and are relatively homogeneous. The Faroe Islands and Iceland are islands in the North Atlantic. The Faroe Islands follow the Danish medical guidelines on antimicrobial use and most physicians practicing in the country have trained in Denmark. Important information on prescribing practices can be obtained by comparing the antibacterial use in these countries and this information can also be used to analyse and compare antibacterial resistance rates in these countries.

The objective of this study was to describe, compare, and analyse the use of J01 systemic antibacterial [3] agents in the Faroe Islands, Denmark, and Iceland in 1999–2011.

Materials and methods

For the period 1999–2011, information on the total sales of antibacterials to humans (including nursing homes and hospitals) was obtained from the National Pharmacist in the Faroe Islands (Richard Schwartson and Ann W. Jensen), from the Icelandic Medicines Agency [4], the Directorate of Health, and NOMESCO (Nordic Medico-Statistical Committee) [5] for Iceland, and the Danish Medicines Agency [6] and DANMAP [7] for Denmark. These data were used to represent the total use of systemic antibacterials (ATC J01, referred to hereafter as antibacterials) expressed in defined daily dosages (DDD; World Health Organization (WHO) Collaborating Centre for Drug Statistics Methodology, Guidelines for ATC classification and DDD assignments [3]) per 1000 inhabitants per day (DID). The DDD assignments for Denmark and the Faroe Islands are based on the 2013 version of the ATC/DDD index, whereas the Icelandic data are based on the assignments for the respective years and were not changed retrospectively. This should not affect our comparisons as no changes in DDDs for antibacterials have been made since 2005 (except for ceftezole, which was not used [3]). Although not all antibiotics/antibacterials sold are consumed, the terms 'consumption' and 'use' are often used instead of the term 'sales' in the following text.

The total antibiotic use (1999–2011) was described for all of the major antibiotic groups according to the ATC classification: tetracyclines (J01A), penicillins (J01C), cephalosporins (J01D), sulphonamides and trimethoprim (J01E), macrolides and lincosamides (J01F), aminoglycosides (J01G), quinolones (J01M), and other antibiotics (J01X).

Age-specific information on the use of tetracyclines and macrolides in the community was based on outpatient prescriptions from pharmacies (Iceland, Directorate of Health; Denmark, DANMAP; Faroe Islands, National Pharmacist).

Results

During 1999–2011, the yearly sale of antibiotics in the Faroe Islands was 16.3–18.6 (mean 17.7 ± 0.385) DID; this was higher than in Denmark at 13.5–19.5 (mean 16.3 ± 1.267) DID, but less than in Iceland at 19.9–23.4 (mean 21.8 ± 0.746) DID. The sale was remarkably stable in the Faroe Islands, but increased steadily in Denmark (from 13.5 to 19.5 DID). In Iceland the sales varied over the years, being lowest in 2001 (19.9 DID) and highest in 2006 (23.4 DID). In Denmark the sales ranged from 14.3 to 16.9 DID during the same period (Figure 1). The sales of the main ATC classes for the y 2005–2011 are displayed in Figure 2.

Tetracycline J01A

The difference in total sales among the 3 countries can be explained largely by the high consumption of tetracyclines in Iceland (5.2 DID). Tetracyclines represented about 24% of the total J01 antibacterial sales in Iceland, compared to 8% and 10% of the total use in the Faroe Islands and Denmark, respectively.

There were differences between the countries in the tetracyclines used, as Iceland used only doxycycline (Figure 3). In all 3 countries, the majority of tetracyclines were prescribed to people in the age

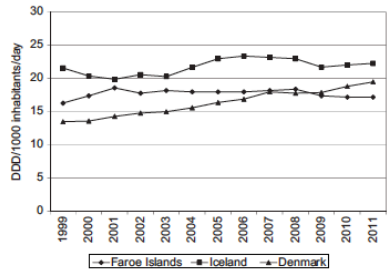

Figure 1. Total sales of antibacterial agents for systemic use (J01) in the Faroe Islands, Iceland, and Denmark, 1999–2011.

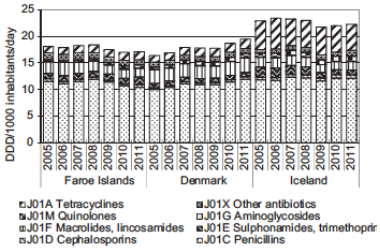

Figure 2. Sales of the main antibacterial groups for systemic use (ATC groups J01A, C, D, E, F, G, M, and X) in the Faroe Islands, Denmark, and Iceland, DDD/1000 inhabitants/day, 2005–2011.

group 15–19 y (3.2, 10.3, and 4.7 DID for Faroe Islands, Iceland, and Denmark, respectively) and in Iceland there was also higher use among those aged >60 y (Figure 4). In Denmark, tetracycline use increased from 1.3 DID in 2005 to 1.8 in 2011.

Penicillin J01C

Although the total sales of penicillins did not differ much between the countries, there was a marked difference in the substances used (Figure 5). Broad-spectrum penicillins alone and in combination with beta-lactamase inhibitors were used much more in Iceland compared with the other countries. During 2005–2011, the mean use of penicillins was 11.8, 11.2, and 10.9 DID in Iceland, the Faroe Islands, and Denmark, respectively.

The mean sales of the broad-spectrum penicillins (J01CA) was 4.1, 2.9, and 3.6 DID, and of the combinations with the beta-lactamase inhibitors (J01CR) was 3.8, 0.2, and 0.5 DID in Iceland, Faroe Islands, and Denmark, respectively.

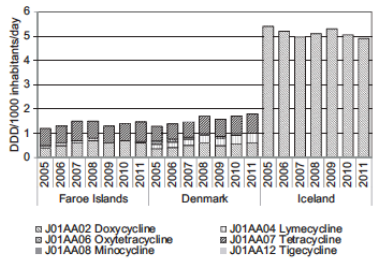

Figure 3. Sales of tetracycline subgroups (ATC J01AA) in the Faroe Islands, Denmark, and Iceland, 2005–2011.

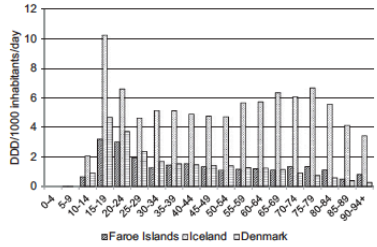

Figure 4. Mean sales of tetracyclines (ATC J01A, outpatient prescriptions) in the Faroe Islands, Denmark, and Iceland for the y 2005–2011, in DDD/1000 inhabitants/day, according to age group.

The mean use of narrow-spectrum penicillins (J01CE) was lower in Iceland than in the Faroe Islands and Denmark, i.e. 2.7, 6.8, and 5.6 DID, respectively (Figure 5).

Macrolides J01FA

The average sale of macrolides was highest in Denmark, followed by the Faroe Islands and Iceland (2.4, 1.9, and 1.6 DID, respectively) (Figure 6). The use of erythromycin declined in all countries during the study period, leading to decreased total macrolide use in the Faroe Islands and Iceland, but not in Denmark because of increasing roxithromycin use. In 2011 the most commonly used macrolides in the Faroe Islands, Denmark, and Iceland were erythromycin (53%), roxithromycin (46%), and azithromycin (54%), respectively.

As DID underestimate antibacterial use in children, the number of macrolide prescriptions was also

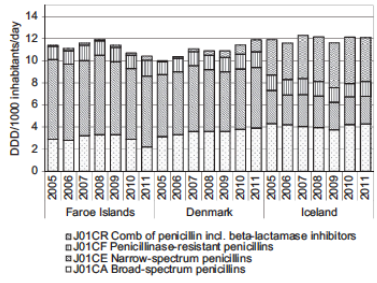

Figure 5. Sales of the main penicillin subgroups (ATC J01CA, E, F, and R) in the Faroe Islands, Denmark, and Iceland, 2005–2011.

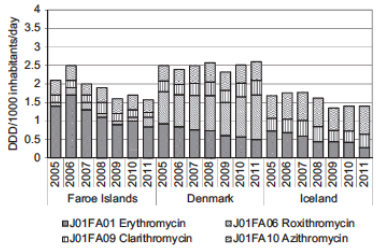

Figure 6. Sales of the main macrolide subgroups (ATC J01FA01, A06, A09, and A010) in the Faroe Islands, Denmark, and Iceland, 2005–2011.

analyzed. In the Faroe Islands and Denmark, erythromycin was mainly prescribed to children in the age group 0–4 y, and in Iceland it was prescribed to people in all age groups. There were marked differences in the use of azithromycin, with very high use in the 0–4 y age group in Iceland (mean 8.453 prescriptions/100,000 inhabitants/y for the y 2005–2011) as opposed to very little use in this age group in the Faroe Islands and Denmark (mean 762 and 1.977, respectively). The pattern of azithromycin use for the other age groups was similar.

Sulphonamides and trimethoprim J01E

The average sale of sulphonamides and trimethoprim was 1.1, 0.9, and 1.4 DID in the Faroe Islands, Denmark, and Iceland, respectively. There was a marked decrease throughout the period in Iceland compared to a relatively constant use in the Faroe Islands. In Denmark sulphonamide use has decreased, while trimethoprim use has increased.

Cephalosporins J01D

The average sales of cephalosporins did not differ much between the countries (0.4, 0.4, and 0.6 DID in the Faroe Islands, Denmark, and Iceland, respectively). The first-generation cephalosporins were not used in the Faroe Islands and to a very small extent in Denmark (mean 0.05 DID). Cephalexin (mean 0.3 DID) was the agent mainly used in Iceland. The second-generation cephalosporins represented the most used subclass in the Faroe Islands (mean 0.3 DID) and Denmark (mean 0.3 DID) and the next most commonly used cephalosporin in Iceland (mean 0.2 DID). Third-generation cephalosporins were first sold in the Faroe Islands in 2008 (mean 0.08 DID). The mean use of third-generation cephalosporins was 0.02 and 0.07 DID in Denmark and Iceland, respectively. No use of fourth-generation cephalosporins was recorded in any country.

Quinolones J01M

The average sale of fluoroquinolones was 0.4, 0.9, and 0.7 DID in the Faroe Islands, Iceland, and Denmark, respectively. The use of fluoroquinolones increased steadily during the period in all 3 countries (0.3 to 0.6 DID in Faroe Islands, 0.5 to 0.8 in Denmark, and 0.8 to 1.05 in Iceland).

Other antibiotics J01X and lincosamides J01FF and aminoglycosides J01GB

The average sales of other antibacterials was 1.1, 0.9, and 0.8 DID in the Faroe Islands, Denmark, and Iceland, respectively. The average sale of nitrofurantoin was 0.8, 0.5, and 0.5 DID in the Faroe Islands, Denmark, and Iceland, respectively, and decreased in the Faroe Islands, as opposed to an increase in Iceland and a constant use in Denmark.

Low use of lincosamides (0.06, 0.13, and 0.0 DID) and aminoglycosides (0.1, 0.3, and 0.1 DID) was registered in the Faroe Islands, Iceland, and Denmark, respectively.

In January 2012, the same 46 antibiotics (J01) were on the market in the Faroe Islands and Denmark: doxycycline, lymecycline, oxytetracycline, tetracycline, tigecycline, ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam, benzylpenicillin, phenoxymethylpenicillin, dicloxacillin, flucloxacillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefalexin, cefuroxime, cefotaxime, ceftazidime, ceftriaxone, aztreonam, meropenem, ertapenem, doripenem, trimethoprim, sulfamethizole, sulfamethoxazole/trimethoprim, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, tobramycin, gentamicin, ofloxacin, ciprofloxacin, moxifloxacin, vancomycin, teicoplanin, colistin, fusidic acid, metronidazole, nitrofurantoin, methenamine, linezolid, and daptomycin [6,7].

In Iceland, there were 31 antibiotics (J01) marketed in January 2012: doxycycline, amoxicillin, pivmecillinam, benzylpenicillin, phenoxymethylpenicillin, dicloxacillin, cloxacillin, flucloxacillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefalexin, cefazolin, cefuroxime, ceftazidime, ceftriaxone, meropenem, ertapenem, trimethoprim, sulfamethoxazole/trimethoprim, erythromycin, roxithromycin, clarithromycin, azithromycin, clindamycin, gentamicin, ciprofloxacin, vancomycin, metronidazole, nitrofurantoin, methenamine, linezolid, and daptomycin [4].

Discussion

Iceland had the highest sales of antibacterial agents, followed by the Faroe Islands and Denmark. Not surprisingly the pattern of antibacterial use in the Faroe Islands was similar to the pattern in Denmark, as most physicians practicing in the Faroe Islands are trained in Denmark. Although most Icelandic doctors receive their training in Iceland, most seek their specialist training abroad, mainly in Sweden, the USA, Norway, Denmark, and the UK. Antibacterial use is higher in the USA than in the Nordic countries, and the fact that a significant proportion of Icelandic doctors train in the USA may be one of the reasons for the higher antibacterial use in Iceland. The total sale of antibiotics was constant in the Faroe Islands, increased in Denmark, and showed minor fluctuations and variations in Iceland.

The difference between the countries was mainly due to tetracycline use, as the sale in Iceland was 3 times higher than that in the Faroe Islands and Denmark. The high tetracycline use in Iceland is due to the high consumption in teenagers and young adults, acne being the most likely indication. Why acne is treated more commonly with tetracyclines in Iceland than in the other countries is not known, and only Finland and the UK come close in tetracycline use [8]. The high use of tetracyclines for young adults reflects long-term treatment for acne rather than repeated courses. Recently, an increase in tetracycline use was reported among young Danish adults. In 2008, they accounted for 4.5 DID (29%) of all antimicrobial agents prescribed to 15–19-y-olds, but by 2011, this had increased to 5.4 DID (33%), of which at least 59% was prescribed for skin disorders (indication not recorded for 21%) [9].

Penicillins were the most frequently prescribed antibiotics in all countries. Phenoxymethylpenicillin was the most commonly prescribed penicillin in the Faroe Islands and Denmark, and the broad-spectrum penicillins with and without beta-lactamase inhibitors in Iceland. This is similar to other European countries, where penicillins were the most frequently prescribed outpatient antibiotics in 2009 [10]. In the Faroe Islands, the use of broad-spectrum penicillins was less than in Iceland and Denmark, possibly reflecting a more conservative antibacterial policy. The high use of broad-spectrum penicillins in Iceland is probably caused by a much higher prevalence of multi-resistant pneumococci in isolates from the middle ear in Iceland than in Denmark and the Faroe Islands, and the increase in recent years is probably due to a switch from normal dosing to high doses of amoxicillin in children [11]. This use is comparable to the J01C consumption in southern Europe [12]. The lower use of broad-spectrum penicillin from 2009 to 2011 in the Faroe Islands can partly be explained by the fact that in March 2011 pivmecillinam (Selexid) was taken off the market because of the association with carnitine transporter deficiency [13,14].

The use of macrolides was lowest in Iceland and highest in Denmark and there were marked differences between individual macrolides. The Faroe Islands mainly used erythromycin, but this agent had to a great extent been replaced by roxithromycin in Denmark and by azithromycin in Iceland. Since azithromycin is normally given in a single daily dose for a short period, its use is more difficult to compare using DDD, especially in children. When the number of azithromycin prescriptions according to age groups was compared, it was most commonly used in the age groups 15–25 y in the Faroe Islands and Denmark and 0–4 y in Iceland. This most likely reflects its main indications, i.e. for chlamydia in the Faroe Islands and Denmark and acute otitis media in Iceland. Official recommendations for the treatment of acute otitis media in Iceland do not include azithromycin, but its ease of use for children attending day care centres has made it popular for this indication. Roxithromycin has been used for some years in Denmark, first because it has fewer side-effects than erythromycin and second because it is cheaper than the other newer macrolides. However, in the last 7 y clarithromycin has been recommended as the standard macrolide due to beneficial pharmacokinetics and the availability of suspension, tablet, and intravenous fluid forms, but for unknown reasons this has not influenced the general use of macrolides (http://www.medicin.dk). The overall use of macrolides remained relatively constant in our study, but in 2011 ESAC reported an increase in use in most European countries from 1997 to 2009 [15].

Sulphonamides and trimethoprim use was a relatively minor part of the total antibacterial consumption in all 3 countries, but the use of the individual agents differed markedly between the countries. Trimethoprim represented roughly half the consumption in this class in all countries, but the sulphonamide consumption in Iceland was solely represented by the combination of trimethoprim/sulfamethoxazole, and mainly by sulphonamides alone in Denmark and the Faroe Islands. Sulphonamides (without trimethoprim) were not available in Iceland.

The use of quinolones increased in all 3 countries during the study period, which is similar to the trend in other European countries [16]. In the group 'other antibacterial agents', nitrofurantoin was used most often in all 3 countries.

A partial explanation for the constant antibacterial use in the Faroe Islands could be that there is no antimicrobial policy and no information on antimicrobial resistance in the country.

Iceland has had the highest consumption of antibacterial agents of the Nordic countries [8,17] and is close to the median of European countries (ESAC yearbook 2009–2010). Iceland was also listed as having the highest consumption of tetracycline in Europe in 2009 [8]. Denmark has, until recently, been one of the lowest consumption countries in Europe, but in 2010 DANMAP reported that Denmark had the highest increase in J01 antibacterial use since the DANMAP program was initiated in 1995 [7]. From DANMAP we know that the dosage (DDD) has been the consumption indicator with the highest increase. The number of patients treated and the number of packages prescribed have not increased to the same degree. National guidelines have been updated recommending different antibiotics and higher dosages for some indications, but this can only explain some of the increased antibacterial use in Denmark, as can demographic factors such as increasing age [18].

Despite the ethnic and cultural similarities between the Faroe Islands, Denmark, and Iceland, there were differences in total use, mainly reflecting high tetracycline use in Iceland, and important differences in the use of individual penicillins and macrolides. Analysing these differences in relation to antibacterial resistance data may give important information with regard to the effects of community antibacterial consumption on antibacterial resistance.

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Declaration of interest: The authors have no conflicts of interest to declare.

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Paper II

Marita Debess Magnussen, Shahin Gaini, Hannes Gislason, Karl G. Kristinsson. Antibacterial resistance in *Streptococcus pyogenes* (GAS) from healthy carriers and tonsillitis patients and association with antibacterial sale in the Faroe Islands. *APMIS,* 2016, 124(4), 327-32.

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Antibacterial resistance in *Streptococcus pyogenes* (GAS) from healthy carriers and tonsillitis patients and association with antibacterial sale in the Faroe Islands

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Magnussen MD, Gaini S, Gislason H, Kristinsson KG. Antibacterial resistance in *Streptococcus pyogenes* (GAS) from healthy carriers and tonsillitis patients and association with antibacterial sale in the Faroe Islands. APMIS 2016; 124: 327–332.

The aim of this study was to investigate the antibacterial resistance of *Streptococcus pyogenes* (GAS), and correlate the findings with the sales of erythromycin and tetracycline. General practitioners in the Faroe Islands were recruited to send oropharyngeal swabs. From an ongoing pneumococcal study, nasopharyngeal swabs were sampled from healthy children 0–7 years of age. Erythromycin susceptibility data from Iceland were obtained from the reference laboratory at the Landspitali University Hospital. Susceptibility testing in the Faroe Islands and Iceland was performed according to CLSI methods and criteria. The resistance rate to erythromycin and tetracycline found in patients in the Faroe Islands in 2009/2010 was 6% and 30% respectively. Tetracycline resistance in patients declined significantly from 2009 to 2010 (37–10%, p-value = 0.006 < 0.05) and differed significantly between age groups (p-value = 0.03 < 0.05). In Iceland, there was a peak in erythromycin resistance in 2008 (44%) and a substantial decrease in 2009 (5%). Although the prevalence of erythromycin and tetracycline resistance in the Faroe Islands and Iceland may be associated with antimicrobial use, sudden changes can occur with the introduction of new resistant clones.

Key words: *Streptococcus pyogenes* (GAS); antibacterial resistance and sale; Faroe Islands; Iceland; Denmark.

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Streptococcus pyogenes (GAS) has the ability to colonize the upper respiratory tract asymptomatically and to cause a wide variety of diseases that differ in severity and tissue tropism. Such diseases range from relatively mild infections, such as pharyngitis and impetigo, to the more severe forms of invasive disease, like streptococcal toxic shock syndrome and necrotizing fasciitis (1, 2). Antibacterial resistance to GAS requires close attention, particularly as the rate of resistance to macrolides continues to increase in Europe (3–6).

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At present, penicillin is the first-line therapy in treatment of most GAS infections. There has not been any report of resistance in GAS to penicillin (2, 7, 8). Macrolides, such as erythromycin, have commonly been used for the treatment of GAS, especially for those who are allergic to penicillin. The prevalence of macrolide resistance has increased in several countries. This has also been observed in samples from asymptomatic, colonized individuals (4, 9–12).

The prevalence of resistance to antibiotics depends in part on their use in the community (13, 14). Some studies have demonstrated a relationship between GAS resistance prevalence and antibacterial

consumption (15, 16). In the Faroe Islands, information about GAS resistance prevalence in the community is lacking. In our previous studies, we showed significant differences in macrolide and tetracycline use between the Faroe Islands, Denmark and Iceland (17). The objective of this study was to examine the prevalence of antibacterial resistance in GAS isolates in the Faroe Islands, compare the findings with Iceland and Denmark, and correlate the findings to antibacterial sales, especially the sale of erythromycin and tetracycline.

MATERIAL AND METHODS

Study 1 (patients)

The Faroe Islands is populated with approximately 49 000 inhabitants, and there are 24 general practitioners (GPs), twelve of which participated in the present study. The GPs were selected with the intent to represent all the 18 islands in the Faroe Islands. They sampled 125 oropharyngeal (throat) swabs from patients with sore throat in all age groups positive in GAS strep test. The sampling period was from 2009 and 2010 (January–March).

Study 2 (carriers)

This study population is from an ongoing pneumococcal study (not published). In 2009 and 2010 (January–March), nasopharyngeal swabs were sampled from 616 healthy children 0–7 years old of age attending day-care centres in the Faroe Islands.

Oropharyngeal and nasopharyngeal swabs

The oropharyngeal samples were obtained with a sterile charcoal medical applicator and the nasopharyngeal samples were obtained with a sterile medical applicator (Statens Serum Institute, Copenhagen, Denmark), stored for a maximum of 24 h before they were inoculated on blood agar containing gentamicin (5 mg/L), a bacitracin disc was placed in the centre (Oxoid, Roskilde, Denmark) and incubated anaerobically. Colonies were identified with conventional methods, that is, colonies with characteristic appearance were identified by bacitracin disc and grouped by Lancefield grouping (ImmuLex™ Streptococcus Group Kit; Statens Serum Institute, Copenhagen, Denmark).

Antimicrobial susceptibility testing

Susceptibility to penicillin, erythromycin, clindamycin and tetracycline was determined by disc diffusion test according to CLSI methods and criteria (18); (discs from Oxoid).

Icelandic data

Erythromycin susceptibility data from Iceland were obtained from the Department of Clinical Microbiology, Landspitali University Hospital (19). It is the reference laboratory for Iceland and covers approximately two-thirds of the Icelandic population and receives samples from both outpatients and inpatients. The data are from all specimens, although the majority of the specimens come from patients with tonsillitis. Susceptibility testing was according to CLSI methods and criteria (18).

Antibacterial use

Information on antibacterial use was obtained from a recently published study (17).

Statistical methods

The statistical analysis was made by using the R-language for statistical computing and graphics (www.r-project.org); and the RStudio integrated development environment (www.rstudio.com).

The antibacterial resistance (R + I) of GAS isolates sampled in 2009 and 2010, respectively, was susceptibility-tested against four antibiotics (penicillin, erythromycin, clindamycin and tetracycline). 2 × 2 contingency tables (not shown) for each tested antibiotic were constructed with counts of resistant and non-resistant samples for the isolates from 2009 as compared to the isolates from 2010. This was done both for patients and carriers; and Fisher's exact test `fisher.test` (20) was used to compare the resistance proportions in 2009 and 2010.

Tetracycline resistance (R + I) of GAS isolates in 2009–2010 was also investigated for possible differences between age groups. To formally test differences between resistances for different age groups, we divide the isolates from different age groups into two resistance groups depending on the magnitude of the observed resistances.

We assume resistances (R + I) in individual age groups to be low, if the group-resistance is below the lower confidence limit for the mean of (R + I). Otherwise, we assume that the individual group-resistance is either similar or higher than the mean. For simplicity, we name these two resistance groups as $\text{low}_{(R+I)}$ and $\text{high}_{(R+I)}$ respectively.

A 2 × 2 contingency table (not shown) was constructed with counts of resistant and non-resistant samples for the isolates in the $\text{high}_{(R+I)}$ group as compared to the isolates in the $\text{low}_{(R+I)}$ group; and Fisher's exact test `fisher.test` (20) was used to compare the resistance proportions in the two resistance groups constructed from different age groups.

Ethical approval

The adult patients gave their personal consent and parents gave consent on behalf of their children. Ethical approval was obtained from the Scientific Ethical Board of the Faroe Islands on 7 November 2008 and the data collection approval was obtained from the Data Protection Agency on 6 June 2008.

RESULTS

There were 169 GAS isolates included in the Faroese study (125 from oropharyngeal swabs and 44 from nasopharyngeal swabs). The mean age of

GAS carriers (children) was 4.4 years and the average age of patients was 25.4 years.

The resistance rate of GAS oropharyngeal and nasopharyngeal isolates tested to the given antibacterial drugs for the years 2009 and 2010 can be seen in Table 1. The resistance rates include both resistant (R) and intermediate (I) isolates.

Study 1 (patients)

For the years 2009 and 2010 combined, the resistance rate in isolates from the oropharyngeal swabs was: penicillin 0%, erythromycin 6%, clindamycin 2% and tetracycline 30%, of these 21% had reduced susceptibility (intermediate) to tetracycline. There was a significant decrease in tetracycline resistance from 2009 to 2010 37% to 10% (Fisher test, p-value = 0.006 < 0.05; Table 1).

Over all age groups, the mean tetracycline resistance of R + I is 38 resistant isolates out of 125 isolates, that is, about 30%, with 23% and 39% as the lower and upper confidence limits, respectively. We find $high_{(R+I)}$ resistances in age groups 0–15 years; $low_{(R+I)}$ for 15–20 years; $high_{(R+I)}$ for 25–40 years; then shifting between $low_{(R+I)}$ and $high_{(R+I)}$ for each year group until it remains low for the remaining groups of 60–75 years. The majority of the tetracycline-resistant and intermediate isolates were found in the age groups 0–15 years (n = 16 out of 47 isolates) and 25–40 years (n = 17 out of 47 isolates).

In total, we find 35 resistant of 100 isolates in the $high_{(R+I)}$ group as compared to three resistant out of 25 isolates in the $low_{(R+I)}$ group. The statistical test rejects that the resistances proportions are equal and independent of our division (`fisher.test`, p-value = 0.03 < 0.05, which is significant at the 95%-confidence level) (Fig. 1).

The mean sale of tetracycline in the Faroe Islands from 2005 to 2012 can be seen in Fig. 2.

The main consumers of tetracycline in the Faroe Islands are people in the age group 15–25 years of age.

Study 2 (carriers)

The susceptibility of GAS nasopharyngeal strains tested to antibacterial drugs for the years 2009 and 2010 combined can be seen in Table 1. The resistance rate was: penicillin 0%, erythromycin 2%, clindamycin 2% and tetracycline 5%. There was no significant change in resistance rates from 2009 to 2010 in any of the tested antibacterials (Table 1).

Study 1 and 2 combined: For the years 2009 and 2010, the resistance rates in the 169 tested oropharyngeal and nasopharyngeal isolates were: penicillin 0%, erythromycin 5%, clindamycin 2% and tetracycline 24% (Table 1).

Of the 169 isolates, clindamycin resistance was only recorded in three isolates and two of them were intermediate (Table 1).

Data from Iceland

The number of isolates tested for erythromycin susceptibility from specimens submitted to the Landspitali University Hospital were 657, 195, 534, 633, 526, 482, 478, 425 for the years 2005–2012 respectively. The susceptibility results can be seen in Fig. 3. The proportion of erythromycin non-susceptible isolates was below 10% for all the years except when it started to increase in 2007, peaking in 2008 (44%), followed by a decline to 5% in 2009 (Fig. 3).

DISCUSSION

Erythromycin resistance among GAS isolates might easily remain unnoticed if physicians are mainly performing rapid antigen tests instead of sending swabs for culture. Even when throat cultures are obtained by GPs, susceptibility testing may not be routinely performed. Most often a positive Strep A test at a GP's office will result in an antibiotic prescription without further culturing and resistance testing. This limits the number of specimens available for culture and therefore the number of

Table 1. Susceptibility testing for penicillin, erythromycin, clindamycin and tetracycline in GAS isolates in the Faroe Islands, 2009–2010

	2009				2010				Total isolates 2009/2010				Diff. 2009–2010	
	Patients		Carriers		Patients		Carriers		Patients	Carriers	Patients/carriers		Patients	Carriers
	n = 95	R + I%	n = 30	R + I%	n = 30	R + I%	n = 14	R + I%	R + I%	R + I%	n = 169	R + I%	<0.05	<0.05
Penicillin	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
Erythromycin	5	5	1	3	3	10	0	0	6	2	9	5	0.40	1
Clindamycin	1	1	0	0	1	3	1	7	2	2	3	2	0.42	0.32
Tetracycline	35	37	1	3	3	10	1	7	30	5	40	24	0.006	0.54

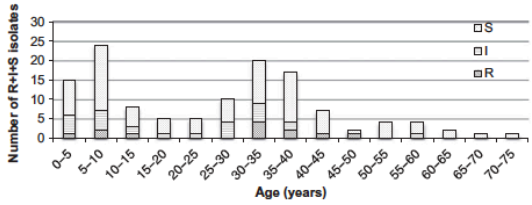

Fig. 1. Number of tetracycline-resistant (R), intermediate (I) and sensitive (S) GAS isolates sampled in 2009–2010 from patients in the Faroe Islands. The stacked bars are plotted for patients aged between 0 and 75 years, and divided into 15 age groups of 5 years size. The height of the bars corresponds to the total number of isolates within each age group and the total number (sample size) of isolates was 125. Tetracycline resistance was significantly associated with particular age groups (R + I) Fisher test: (p-value = 0.03 < 0.05).

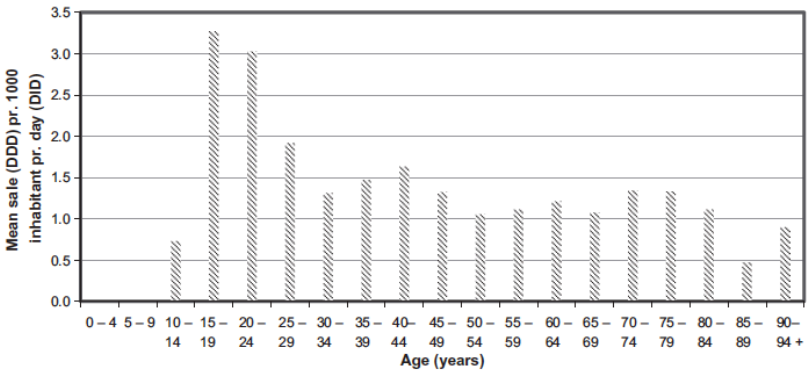

Fig. 2. Mean sale of tetracycline (ATC J01A, outpatient prescriptions) in the Faroe Islands for the years 2005–2012, in DDD/1000 inhabitants/day, according to age (DID).

isolates available, an important limitation for this study. The study gives important information about the resistance rates among GAS isolates in the Faroese community for the study period, however, the period was not long enough to give significant information about time trends. In both study populations, the resistance rates to erythromycin were low and similar to the resistance rates found in Denmark and lower than in Iceland (8, 19), despite lower macrolide use in Iceland than in the Faroe Islands and Denmark (17). It is possible that this is because azithromycin is the most commonly used macrolide in Iceland, and long-acting macrolides are more likely to induce resistance (21).

Studies from Finland, Germany and France and other countries have shown a decreased level of macrolide resistance by reducing the use of macrolides (22–26). Therefore, the decreasing sale of erythromycin and low use of azithromycin in the Faroe Islands and Denmark (17) can possibly explain the low erythromycin resistance rate. The sudden increase in erythromycin resistance in Iceland from 5% in 2006, to 44% in 2008, with a decline to 5% in 2009, without any interventions, suggests a rapid spread of a new erythromycin resistant clone in 2007–2008, followed by its virtual disappearance in 2009. The rapid increase and decline in erythromycin resistance in Finland from 1992 to 1996 were, in retrospect, considered to be clonally related, suggesting that rapid changes in susceptibility rates are more related to introductions of new clones rather than to antibiotic use (23, 27). A study from Canada showed a higher macrolide resistance rate in children than in adults (22), how-

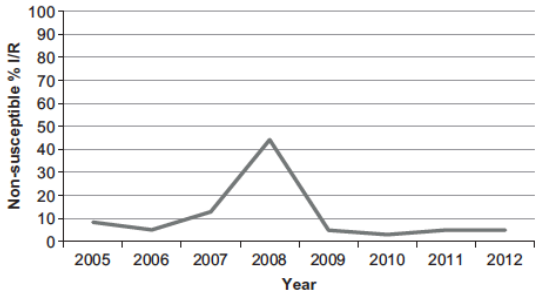

Fig. 3. Erythromycin resistance rate of GAS isolates submitted to the clinical microbiology laboratory at the Landspitali University Hospital, 2005–2012.

ever, this difference could be because of a higher macrolide use in children and the main users of erythromycin in the Faroe Islands were children in the age group 0–4 years of age (17). However, there was no significant difference in the erythromycin resistance rate among children and patients in the Faroe Islands. This could perhaps reflect that the total macrolide consumption among children and adults has an influence and not only the consumption of macrolides in certain age groups.

A previous study (17) shows a low sale of tetracycline in the Faroe Islands and none interventions has taken place to lower the tetracycline use in the Faroe Islands. Therefore, there is a possibility that the decline in tetracycline resistance from 2009 to 2010 in the Faroe Island is also partly clonally related, as seen in Iceland. In Denmark, tetracycline resistance is relatively high and macrolide resistance is low (28). The same level of tetracycline resistance rate is reported in Iceland (19), but in Iceland the tetracycline resistance rate is increasing and this correlates with the high sale of tetracyclines in Iceland. The main difference in antibacterial use between the Faroe Islands, Denmark and Iceland was the higher use of tetracycline in Iceland (17). The main consumers of tetracycline in the Faroe Islands are people in the age group 15–25 years of age, and in this study the majority of tetracycline resistant and intermediate isolates were found in children (0–15 years) and in people in the age-group 25–40 years old. Although, there was a significant difference in tetracycline resistance between age groups, it is unlikely that this is due to differences in tetracycline use as one of the highest resistance rates was in the youngest age group hardly using any tetracycline. A more likely explanation could be that tetracycline-resistant isolates are being transmitted from older family members. Since the Faroe Islands have a population of only 49 000 people, it is possible that one or very few clones can be circulating in the community at any one time. A study on the clonality of GAS in the Faroe Islands would be of interest.

Macrolide and clindamycin resistance in Faroese GAS isolates was low and moderate to tetracyclines. This may be due to relatively restricted antimicrobial use, yet significant changes may take place with the introduction of new clones resistant to these antibiotics. The current antibiotic recommendations are still relevant. Penicillin as the first line of treatment and macrolides in cases of penicillin allergy. However, macrolide use should not increase since there is a cross-resistance among the macrolide (28).

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TRANSPARENCY DECLARATIONS

The authors have no interests to declare.

AUTHORS' CONTRIBUTIONS

MDM is the PhD student, she planned the study, wrote the manuscript, collected data, analysed data. KGK is the supervisor in the PhD study, he planned the study, wrote the manuscript and analysed data. SG has supervised the work and wrote the manuscript. HG has planned and done the sta-

tistical work together with MDM. All authors read and approved the final manuscript.

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Paper III

Marita Debess Magnussen, Hannes Gislason, Shahin Gaini, Karl G. Kristinsson. Antibacterial Susceptibilities of *Escherichia coli* from Community-Acquired Urinary Tract Infections in the Faroe Islands, Associations with Antibacterial Sales, and Comparison with Iceland and Denmark. *Microb Drug Resist,* 2018 Jan/Feb; 24(1): 40-47. Epub 2017 May

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Antibacterial Susceptibilities of *Escherichia coli* from Community-Acquired Urinary Tract Infections in the Faroe Islands, Associations with Antibacterial Sales, and Comparison with Iceland and Denmark

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Currently, data on *Escherichia coli* antibacterial susceptibilities in the Faroe Islands are lacking. The aim was to investigate the antibacterial susceptibilities of *E. coli* from patients with community-acquired urinary tract infections in the Faroe Islands, correlate with antibacterial sales, and compare with Iceland and Denmark. From 2009 to 2010 and in 2012, 12 general practitioners from the Faroe Islands were recruited to provide urine samples from patients. Antibacterial susceptibilities were determined by disc diffusion testing according to the Clinical and Laboratory Standards Institute methods and criteria. Logistic regression (quasibinomial) of the antibacterial resistance proportions versus mean sales during the period of 2008–2011 was used to determine association. Nonsusceptibility to at least 1 of the 14 antibacterial drugs investigated was found in 54% of the *E. coli* isolates and was most common to ampicillin (46%), followed by sulfamethoxazole (39%), trimethoprim (27%), trimethoprim/sulfamethoxazole (27%), and <10% to the remaining 10 antibiotics. The resistance prevalence did not change significantly with time. From logistic regression modeling, we find significant associations between antibacterial mean sales and antibacterial resistances. For the resistances in the Faroe Islands compared with data from Denmark and Iceland, we infer two groups of resistances indicating different responses—one steep and one gradual—to antibacterial sales. For these two groups, we find $\beta_1 = 4.77$ (Std. Error = 0.624, p-value = 0.002) and $\beta_1 = 0.26$ (Std. Error = 0.020, p-value = 4e-7) for the steep and gradual groups, respectively. This knowledge can potentially be used to predict and control the future increase in *E. coli* resistance with antibacterial sales.

Keywords: *Escherichia coli*, community-acquired UTIs, Faroe Islands, Iceland, Denmark, antibacterial resistance

Introduction

An increase in the frequency of antimicrobial resistance in *Escherichia coli* (*E. coli*), the most common pathogen in urinary tract infections (UTIs), is occurring globally.[1–3] Furthermore, the development of antimicrobial resistance in *E. coli* influences the efficacy of antibiotic treatment for UTIs. The rates of resistance to aminopenicillins and fluoroquinolones are increasing and many isolates have already become multiresistant.[4,5]

Mecillinam/pivmecillinam is among the first-line antibiotics prescribed for uncomplicated UTIs in the Nordic countries. In Faroe Islands and Denmark, sulfamethizol is also used routinely.[6] The use of pivmecillinam in the Faroe Islands is complicated by a high prevalence of a rare genetic disease, primary carnitine deficiency (PCD). Use of pivmecillinam and pivampicillin increases the risk for arrhythmia and cardiac arrest in PCD patients and is therefore contraindicated.[7] Therefore, pivmecillinam was withdrawn from the Faroese market in 2011[8] and sulfamethizol has remained the first-choice antibiotic for community-acquired UTIs since 1948. In 2013, pivmecillinam was reintroduced as an option for treating UTIs in the Faroe Islands, but only for patients where PCD has been excluded.[9]

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Physicians in the Faroe Islands have not routinely sent urine samples from patients with suspected UTIs for culture. Therefore, information on the antibacterial susceptibilities of the bacteria causing community-acquired UTIs in the Faroe Islands is lacking.

Studies on the antimicrobial consumption and association with antimicrobial resistance among *E. coli* are available in the neighboring countries of Iceland and Denmark. Resistance to fluoroquinolones has continuously increased along with increasing ciprofloxacin use.[10,11] In some European countries, the resistance in community-acquired *E. coli* isolates is also increasing.[12]

The objective of this study was to examine the antibacterial susceptibility rates among *E. coli* isolates from outpatients with community-acquired UTIs, determine if susceptibility rates correlate with antibacterial sales in the Faroe Islands, and do similar analyses on comparable data from Iceland and Denmark.

Materials and Methods

Microbiology

The participating general practitioners (GPs) deposited the urine samples on a dipslide containing CLED and MacConkey medium (Oxoid, Roskilde, Denmark). Within 24 hours, they were incubated under aerobic conditions at 35°C for 18–24 hours. Colonies with a minimum count of 100,000/ml were taken from the MacConkey part of the dipslide and inoculated on 5% sheep blood agar and incubated at 35°C for 18–24 hours. Typical colonies that were oxidase negative and indole positive were considered to be *E. coli*.

Susceptibility to ampicillin, cefoxitin, trimethoprim, trimethoprim/sulfamethoxazole, nitrofurantoin, mecillinam, cefpodoxime, amoxicillin/clavulanic acid, cefuroxime, ceftriaxone, nalidixic acid, gentamicin, ciprofloxacin, and sulfonamide (sulfamethizol) (Oxoid, Roskilde, Denmark) was determined by disc diffusion test according to the CLSI methods and criteria.[13]

Data from Iceland and Denmark

Susceptibility data from Iceland were obtained from the Department of Clinical Microbiology, Landspitali University Hospital.[14] Susceptibility testing was performed according to the CLSI methods and criteria.[13] Susceptibility data from Denmark were obtained from Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP).[11,15] The isolates tested for susceptibility in Iceland are not given in total isolates and some of the isolates are tested for one, two, or several antibiotics. Therefore, we do not have the total number of tested isolates per year. Susceptibility testing in this article is for resistant (*R*) and intermediate (*I*) isolates in the Faroe Islands, but when comparing Faroe Islands, Iceland, and Denmark, we are using only *R* isolates.

Antibacterial sale

We use sale data to represent the approximate antibiotic consumption. Information on antibacterial sales for the Faroe Islands was obtained from a recently published study[16] and from the National Pharmacist in the Faroe Islands (Richard Schwartson and Ann W. Jensen). DANMAP was the source for information from Denmark.[1,15,17] Information for Iceland was collected from the Icelandic Medicine Agency.[18] We correlated antibacterial sales from 2008 to 2011 with data on antibacterial resistance for 2009, 2010, and 2012. We believe that it is reasonable to have a lag time of 1 year since others have observed that the change in resistance may be followed 1 year or more after the antibacterial sale.[19,20]

Statistical analysis

The statistical analysis was performed by using the R language for statistical computing and graphics (www.r-project.org) and the RStudio integrated development environment (www.rstudio.com).

Differences between the years 2009/2010 and 2012 in the Faroe Islands

Fisher's exact test, *fisher.test*,[21] was used to test for significant differences in resistances. A 2×2 contingency table was constructed for each antibiotic with counts of nonsusceptible and susceptible isolates from two population samples (2009/2010 and 2012) taken in the Faroe Islands. Isolates sampled in 2009 ($n=27$) and 2010 ($n=63$) were combined to 2009/2010 ($n=90$) and the antibacterial resistance of *E. coli* against 14 antibiotics was compared with the corresponding resistance of 120 isolates sampled in 2012. Binomial confidence intervals for the 14 antibacterial mean resistances of the total sampled isolates (210) were constructed by using *binom.confint* with methods = "exact" from the R package *binom*.

Test for trend in antibacterial resistance for the Faroe Islands

Data for antibacterial sales in the Faroe Islands for the 4 years, 2008–2011, were found for 7 of the 14 antibiotics. The variability of the mean resistances of the 7 antibiotics was investigated for any linear dependence on different mean sales. The test for trend in proportions *prop.trend.test*[21] was used, which in our case, is a weighted linear regression of the resistances on ordered sales categories—represented by group scores. The test was used to determine if there was no trend (zero slope) in resistance compared with sales (group scores).

Logistic modeling of antibacterial resistance with sales

Logistic regression[21] was used to accurately model the dependency between resistance and antibiotic sales in the Faroe Islands, Iceland, and Denmark. Measurements with two possible values (nonsusceptible, susceptible) are modeled. To constrain the resistance proportions R between 0 and 1, they are transformed to log $[R / (1-R)]$ = log odds R. We used a simple linear model: log odds $R_i = \beta_0 + \beta_1 x_i$ for the resistance R_i depending only on one continuous predictor variable x_i representing antibiotic sales and β_0 and β_1 representing the intercept and slope, respectively. Similarly, we also modeled resistance proportions $R+I$, but in this case, only for the Faroe Islands.

Overdispersion ($\varphi > 1$) indicates nonoptimal fit between the applied logistic model and the data; this may influence the p-values of the model parameters β_0 and β_1. To revise p-values in cases of overdispersion—in the quasi-likelihood

approach—the model parameters are estimated from the ordinary logistic model, but the binomial variance is scaled to $\varphi R_i(1-R_i)/n_i$ by the dispersion parameter φ, standard errors are scaled by $\varphi^{1/2}$, and dispersion is estimated by the X^2 fit statistic: X^2/df for the binomial model.[22]

Antibacterial nonsusceptibility (R+I) versus sales in the Faroe Islands

Logistic models can also be modified to minimize overdispersion. To investigate this effect for the seven data points from the Faroe Islands, we limited the analysis to the sales range where we expect the binomial logistic model to apply. In this part of the study, we assumed that the resistance is approximately independent of sales for low and high antibiotic sales, but follows the binomial logistic model at intermediate sales. We found the sales limits by repeating the logistic regression for different sales values for ampicillin (AMP) until we get minimal overdispersion and stop the iterative process of the most extreme sales data while keeping the resistances unchanged. If large overdispersion still persists, we also excluded as few data points as possible to reduce overdispersion to a low level ($1<\varphi<3$), which we then corrected for using family=quasibinomial rather than family=binomial in the glm function calls of the R software.[23]

Antibacterial resistances versus sales from Faroe Islands, Iceland, and Denmark

Modeling antibacterial resistances for Faroe Islands is complicated by the few (7) data points. Therefore, the data on antibacterial resistances *(R)* versus sales from Faroe Islands 7, Iceland 6, and Denmark 5 were combined into 18 paired vales of antibacterial resistances and mean sales. The *cor.test* function in the R software was used to test for correlation[21] between resistance and sales in the combined sample. The *cor.test* was used as a simple test for correlation using all 18 data points without removing any outliers. In addition, this test does not apply any sample size information used to compute the resistance proportions—as opposed to the trend test and logistic regression—that need the sample size information.

For logistic regression on the combined sample, we assumed that the sample sizes were identical to the sample from Faroe Islands ($n=210$). The sample size assumption gives equal weight to the resistances sampled in each country and no iteration of extreme sales data was applied to the combined sample. Otherwise, we applied similar procedures for the logistic regression as were applied to the sample from Faroe Islands.

Logistic modeling using the R software

We used *glm* function calls of the R software, where dispersion is explicitly observed in the output of quasibinomial logistic regression. The *p*-values <0.05 are considered significant for the model parameters, β_0 and β_1, obtained from the quasibinomial logistic regression when $\varphi>1$.

The R package *visreg* was used on the output from *glm* function calls to calculate the regression curves with lower and upper confidence limits for desired continuous *x*-intervals. Finally, the R package *ggplot2* was used to visualize the results of the regression as solid lines and curves with confidence bands.

Ethical and data protection approvals

Ethical approval was obtained from the Scientific Ethical Board of Faroe Islands on the 7th of November in 2008 and the data collection approval was obtained from the Data Protection Agency on the 6th of June in 2008.

Results

Study population

The Faroe Islands have a population of only 50,000. During the time period 2009–2012, there were 24 GPs, 12 of whom participated in the study. The GPs were selected with the intent to represent all the geographical regions of the Faroe Islands. The GPs sampled mid-stream urine specimens from patients in all age groups whose urine was positive in a dip strip test for leukocytes, nitrate, or both. The sampling period was from January 20th to March 25th 2009 and January 6th to March 25th 2010. The GPs obtained written informed consent from the patients and asked for a urine sample. From June 2010 to December 2010 and April 2012 to December 2012, additional *E. coli* isolates from patients with suspected community-acquired UTIs were also included in our study. The isolates from 2010 to 2012 were collected at the GP clinics, but processed in the department of clinical microbiology at the National Hospital of the Faroe Islands. While the 12 GPs represented all the geographical regions of the Faroe Islands, an increase in the percentage of samples originating from the capital district, Tórshavn, where 41% of the Faroese population lives, was seen over time.[24]

E. coli was determined to be the cause of UTIs in 210 patients. Of those 210 patients, 178 (85%) were women and 32 (15%) were men. During the sampling period from January to March 2009, there were 27 *E. coli* strains isolated from the tested samples. From January to December 2010, 63 strains were isolated. From January to December 2012, 120 strains were isolated.

There was no significant difference found for any of the 14 antibacterial resistances when the data for years 2009/10 and 2012 from the Faroe Islands were compared (Table 1, *p*-values not shown). The resistance rates were highest for the following 4 antibiotics: ampicillin (46%), sulfonamide (39%), trimethoprim (27%), and sulfamethoxazole/trimethoprim (27%), while the resistance of the remaining 10 antibiotics was below 10% (Table 1, Total isolates).

For the 7 antibiotics: gentamicin (CN), co-amoxiclav (AMC), ciprofloxacin (CIP), nitrofurantoin (F300), mecillinam (MEL), sulfonamide (S3), and ampicillin (AMP), the number of resistant (*R*) isolates of $n=210$ was 4, 13, 6, 9, 1, 82, and 96, respectively (Table 1, Total isolates); ordered by the mean sales of 0.13 aminoglycosides, 0.22 amoxicillin and enzyme, 0.47 quinolones, 0.73 nitrofuran, 0.88 pivmecillinam, 1.20 sulfa/trim and 11.1 penicillins, in units of Defined Daily Dose (DDD)/1,000 inhabitants/day (DID) (Table 2). The test for trend (*prop.trend.test*) in resistance proportions *(R)* with antibacterial mean sales using default scores (1–7) is significant (*X*-squared = 231, *p*-value <2.2e-16). Similarly, for the 210 isolates and the same 7 antibiotics ordered by mean sales, the number of resistant isolates *(R+I)* was 4, 51, 7, 23, 16, 83, and 131. The *prop.trend.test* applied to resistance proportions *(R+I)* with antibacterial mean sales using default scores (1–7) is again significant (*X*-squared = 208, *p*-value <2.2e-16).

TABLE 1. SUSCEPTIBILITY TESTING OF *ESCHERICHIA COLI* ISOLATES FROM COMMUNITY-ACQUIRED URINARY TRACT INFECTIONS IN THE FAROE ISLANDS FOR THE YEARS 2009, 2010, AND 2012

					Total isolates			
	2009/2010		*2012*				*95% CI*	
	n=*90*	R%	n=*120*	R%	n=*210*	R%	*Lower*	*Upper*
Ampicillin	39	43	57	48	96	46	39	53
Cefoxitin	2	2.2	2	1.7	4	1.9	0.5	4.8
Trimethoprim	23	26	34	28	57	27	21	34
Trim/Sulfa	23	26	33	28	56	27	21	33
Nitrofurantoin	3	3.3	6	5.0	9	4.3	2.0	8.0
Mecillinam	1	1.1	0	0	1	0.5	0	2.0
Cefpodoxime	2	2.2	4	3.3	6	2.9	1.1	6.1
Co-amoxiclav	5	5.6	8	6.7	13	6.2	3.3	10
Cefuroxime	1	1.1	2	1.7	3	1.4	0.3	4.1
Ceftizoxime	0	0	0	0	0	0	0	1.7
Nalidixic acid	4	4.4	7	5.8	11	5.2	2.6	9.2
Gentamicin	2	2.2	2	1.7	4	1.9	0.5	4.8
Ciprofloxacin	3	3.3	3	2.5	6	2.9	1.1	6.1
Sulfonamide	33	37	49	41	82	39	32	46

Isolates sampled in 2009 ($n=27$) and 2010 ($n=63$) are combined to 2009/2010 ($n=90$) and the antibacterial resistance against 14 antibiotics is compared with the corresponding resistance of 120 isolates sampled in 2012. The resistance of the total sampled isolates ($n=210$) is also shown together with both lower and upper confidence limits.
CI, confidence interval.

The quasibinomial logistic regression for the 7 antibiotic resistance proportions ($R + I$) versus mean sales shows high overdispersion ($\varphi \approx 31$) and an insignificant p-value (0.05) for the sales parameter β_1. Therefore, to minimize overdispersion, we used a maximum sales limit of 1.57 for ampicillin instead of 11.1, and excluded the two data points for co-amoxiclav and mecillinam, considered to be outliers. The revised quasibinomial logistic regression is shown in Figure 1.

Faroe Islands, Iceland, and Denmark

The *cor.test* function in the R software showed both significant Pearson's product–moment correlation (cor.= 0.78, p-value=0.0001) and Spearman's rank correlation (rho=0.68, p-value=0.002). The correlations were found between resistance and mean sales in the combined sample with 18 paired values of antibacterial resistances (R) and mean sales from the Faroe Islands, Iceland, and Denmark (Tables 2 and 3).

Logistic regression on this group of data is again highly overdispersed (≈ 30), but we identify two subgroups of steep and gradual increasing resistances, respectively. By gradual and steep developing antibacterial resistances, we mean that the antibacterial resistances that follow the logistic line with the small slope will typically show lower increase in resistance with sales, while the resistances that follow the logistic line with the large slope will typically show a higher increase in resistance with sales.

TABLE 2. ANTIBACTERIAL SALES FOR SYSTEMIC USE, J01C, J01CA01, J01CA04, J01CA08, J01CR02, J01E, J01EB, J01EA, J01EE, J01 M, J01G, AND J01XE IN FAROE ISLANDS, ICELAND, AND DENMARK IN THE YEARS 2008, 2009, 2010, AND 2011, IN DID

	Antibacterial sales											
	Faroe Islands				*Iceland*				*Denmark*			
	2008	*2009*	*2010*	*2011*	*2008*	*2009*	*2010*	*2011*	*2008*	*2009*	*2010*	*2011*
Penicillins J01C	11.9	11.4	10.7	10.4	12.2	11.6	12.1	12.1	10.9	10.9	11.4	11.9
Ampicillin J01CA01	0.1	0.1	0.1	0.1			0.0		0.1	0.1	0.1	0.1
Amoxicillin J01CA04	1.6	1.4	1.4	1.8	3.4	3.1	3.4	3.4	1.5	1.3	1.4	1.4
Pivmecillinam J01CA08	1.1	1.2	1.0	0.2	0.6	0.7	0.8	0.9	1.7	1.8	1.9	2.0
Amoxicillin and enz. J01CR02	0.1	0.2	0.2	0.4	4.0	4.1	4.2	4.0	0.4	0.6	0.8	1.0
Sulfa/trim J01E	1.1	1.1	1.1	1.5	1.6	1.1	0.9	1.0	0.9	0.8	0.9	0.8
Sulfonamides J01EB	0.4	0.4	0.4	0.6	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.2
Trimethoprim J01EA	0.6	0.7	0.7	0.8	0.8	0.5	0.4	0.5	0.5	0.5	0.5	0.5
Trim/sulfa J01EE	0.0	0.0	0.1	0.0	0.8	0.6	0.5	0.5	0.0	0.0	0.1	0.1
Quinolones J01 M	0.4	0.4	0.5	0.6	0.9	0.9	1.0	1.1	0.8	0.8	0.8	0.8
Aminoglycosides J01G	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1
Nitrofuran J01XE	0.8	0.7	0.7	0.7	0.4	0.5	0.6	0.6	0.5	0.5	0.5	0.5

DID, Defined Daily Dose/1,000 inhabitants/day.

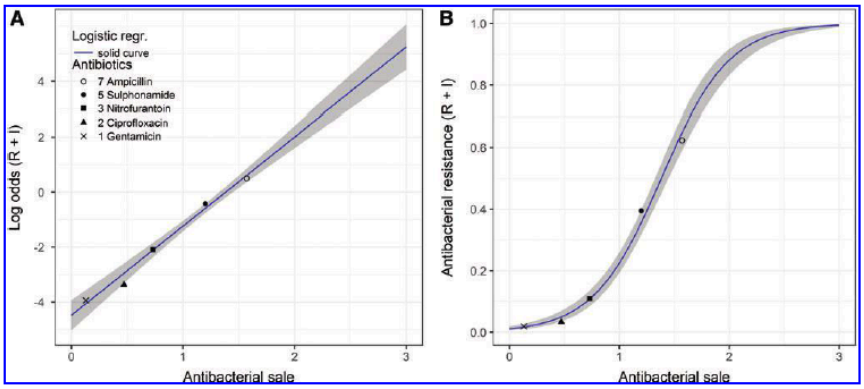

FIG. 1. Logistic regression of five antibiotic resistance proportions in the Faroe Islands versus mean sales 2008–2011, defined daily doses per 1,000 inhabitants per day. The plot **(A)** is on the log odds scale and the plot **(B)** is on the probability scale. Both resistant isolates *R* and intermediates *I* are included. We find $\beta_1 = 3.24$ for slope (Std. Error = 0.226, *p*-value = 0.0007). Overdispersion (≈ 31) when using all seven data points was minimized (≈ 1) by excluding mecillinam and co-amoxiclav and by changing (iterating) from the high sale 11.1 used for ampicillin to 1.57. Color images available online at www.liebertpub.com/mdr

The 18 data points were first plotted and modeled on the logistic scale. It was clear from this plot and model that they could not be well modeled by a single line due to high overdispersion.

Visually, however, this plot also indicated that two different lines possibly could model two different subsets of the data. Initially, this was done manually by looking at the plot with a ruler. Next, the proper logistic modeling was done separately on these two subsets of data.

About 78% of the data points, that is, 14 of 18 resistances—6 of 7 in the steep group (ciprofloxacin, nitrofurantoin, and sulfonamide) and 8 of 11 in the gradual group (ampicillin, co-amoxiclav, gentamicin, and mecillinam)—follow the group's logistic lines, respectively (Figs. 2 and 3).

Discussion

In the Faroe Islands, most uncomplicated UTIs are treated with short courses of empirical antibiotics. Susceptibility data have not been considered when these treatments were prescribed. Our study demonstrates a trend and correlation between antibacterial resistance and antibiotics sales, a finding that is consistent with other published studies on high antibacterial sales and resistance.[25]

Our study also suggests both gradual and steep developing antibacterial resistances, which can potentially be used to predict and control the future increase in *E. coli* resistance with antibacterial sales. However, at this stage—with this small dataset—we should be careful not to claim this effect too strongly to hold in general for other countries, but more like a suggested effect observed locally in this small subset of Nordic countries (Figs. 2 and 3). This relatively good agreement, although not perfect, we believe is good enough to suggest that these effects be closely investigated in future studies. We can hypothesize that the resistance rate of ciprofloxacin in the Faroe Islands will increase to the same level as in Iceland if the sale of the antibiotic in the two countries is equalized (Fig. 2). According to this model, we

TABLE 3. *E. COLI* RESISTANCES (ONLY *R* ISOLATES) IN FAROE ISLANDS, ICELAND, AND DENMARK IN THE YEARS 2009, 2010, AND 2012

	Antibacterial resistance %								
	Faroe Islands			*Iceland*			*Denmark*		
	2009	*2010*	*2012*	*2009*	*2010*	*2012*	*2009*	*2010*	*2012*
Ampicillin	44	43	48	45	47	44	42	40	40
Mecillinam	0	2	0	5	4	6	5	6	6
Amoxicillin/clav acid	0	8	7	7	9	17	NA	NA	NA
Sulfonamide	30	40	41	NA	NA	NA	38	37	33
Ciprofloxacin	7	2	3	11	14	12	11	11	10
Gentamicin	4	2	2	7	7	6	NA	3	NA
Nitrofuran	7	2	5	2	2	2	NA	NA	NA

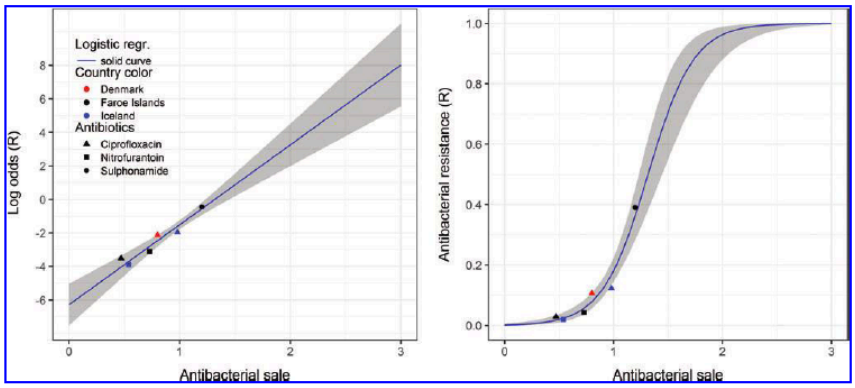

FIG. 2. Logistic regression of six steep increasing antibiotic resistance proportions *R* for three antibiotics (ciprofloxacin, nitrofurantoin, and sulfonamide) versus mean sales 2008–2011. Overdispersion is moderate (≈ 2), and we find $\beta_1 = 4.77$ (Std. Error = 0.624, *p*-value = 0.002). Sulfonamide in Denmark was excluded as an influential outlier. Color images available online at www.liebertpub.com/mdr

could also hypothesize that by staying under the sale of 0.5 DID, the resistance rate would be low and between 0.5 and 1.0 DID, the resistance would be below 20% and >1.5 DID, and the resistance rate would be over 70% (Fig. 2). Figure 2 shows a strong increase in resistance with sales in this model and the high slope could have a large impact on resistance levels with sales as seen in other studies.[26]

There was a high level of resistance prevalence to sulfonamides in the Faroe Islands in 2012 (41%). This is concerning, especially when compared with Denmark, where the resistance prevalence has been decreasing since 2010 to a reported 32% in 2014.[27] Sulfonamides have been on the market for decades. In fact, sulfonamides are still considered the first-choice treatment for uncomplicated UTIs in the Faroe Islands. The sale of sulfonamides has been relatively constant in the Faroe Islands over the last years compared with the declining sale of this antibiotic in Denmark.[11,16]

The resistance prevalence of sulfamethoxazole/trimethoprim and trimethoprim was nearly 30% in the Faroe Islands. A study published by G Kahlmeter[28] on the relationship between antimicrobial usage and resistance in community-acquired *E. coli* found the resistance prevalence of sulfamethoxazole/

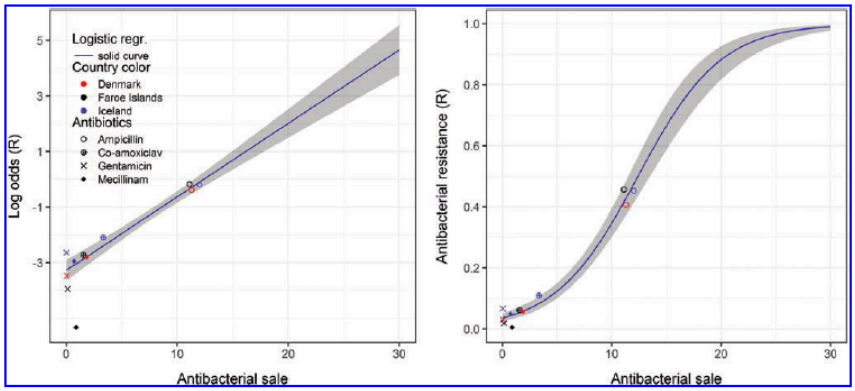

FIG. 3. Logistic regression of 11 gradual increasing antibiotic resistance proportions *R* for 4 antibiotics (ampicillin, co-amoxiclav, gentamicin, and mecillinam) versus mean sales 2008–2011. Overdispersion is again moderate (≈ 2), and we find $\beta_1 = 0.26$ (Std. Error = 0.020, *p*-value = 4e-7). About 8 of these 11 resistances are within the confidence limits of the logistic regression. Color images available online at www.liebertpub.com/mdr

trimethoprim to be 8.2–8.3% in Denmark and Sweden, respectively. The highest resistance prevalence was found in Portugal (26.7%); however, this study was conducted in 2003. The sale of sulfamethoxazole/trimethoprim in the Faroe Islands is quite low, and the drug is new on the Faroese market. Sulfamethoxazole/trimethoprim is not used for community-acquired infections in the Faroe Islands.[9] The high resistance prevalence regarding sulfamethoxazole/trimethoprim in the Faroe Islands is probably due to the constant use of sulfonamides and the relatively high use of trimethoprim.

Our study places ciprofloxacin into the steep increasing resistance group, and fluoroquinolones are not recommended for uncomplicated UTIs due to the risk of increased resistance.[29] Additionally, there were concerns about a higher use of ciprofloxacin and a higher level of resistance to ciprofloxacin in the Faroe Islands. This concern was due to the withdrawal of pivmecillinam and pivampicillin from Faroese market in 2011 to avoid risks associated with PCD.[7,8] The sale did increase as it did in Denmark and Iceland[16]; however, the resistance rate to ciprofloxacin was low in the Faroe Islands compared with Denmark[11] and Iceland.[10,30] DANMAP reported a slightly increased resistance in *E. coli* community-acquired isolates to ciprofloxacin in 2012.[11] In Iceland, the resistance to ciprofloxacin increased steadily until 2010, after which it has remained stable.[30] The increased sale of fluoroquinolones in the Faroe Islands, Denmark, and Iceland may be explained by availability of low price generic ciprofloxacin.

Mecillinam was placed in the gradual increasing resistance group in our study and mecillinam is used to treat uncomplicated UTIs in most Nordic countries.[29] In this study, we found a low level of mecillinam resistance compared with the mecillinam resistance prevalence in Iceland and Denmark.[12,30] However, there is an indication of increased intermediate isolates in the Faroe Islands from 2009/10 to 2012. The low nonsusceptibility in the Faroe Islands is most likely due to the withdrawal of pivmecillinam from the Faroese market in 2012[8] and the decrease in sale of pivmecillinam from 2008 to 2011, directly preceding the withdrawal. However, the amount of pivmecillinam sold was similar in Iceland and the Faroe Islands.

Compared with the high resistance rates for other antibiotics, we found a relatively low prevalence of resistance to amoxicillin clavulanic acid. However, the resistance was higher than in Denmark[31] and lower than in Iceland.[14] The higher resistance in Iceland can be explained by the higher use of penicillins (ATC J01C) and the higher use of amoxicillin with clavulanic acid. Amoxicillin with clavulanic acid has greater activity than amoxicillin without clavulanic acid for bacteria where resistance is caused by beta-lactamase production.[32]

Previously, for *(R+I)* for the Faroe Islands, we assumed that the resistance was approximately independent of sales for extreme antibiotic sales, but followed the binomial logistic model at intermediate sales. We based our assumption on the premise that at low levels of antibiotic sales, there may be insufficient selection pressure to significantly affect the antibiotic resistance. In addition, when high antibiotic sales are due to a popular antibiotic in use for a long period of time, the present time selection effect might now be small. In this case, the resistance could have saturated at lower sales, than present time sales, and not continued to increase with increased sales.

The trend tests applied to the data from the Faroe Islands find significant linear relationships between resistance and sales without using any sales limits or excluding data points. While the linear relationship is important, the ability to accurately predict the future developments in resistance with increased sales using the logistic curves is the major advantage of logistic modeling. The gradual and steep resistance groups were discovered when the small set of resistance data *(R)* from the Faroe Islands was combined into a larger sample with similar data from Iceland and Denmark. In this case, the extreme sale of ampicillin was not iterated to lower values, but instead used and modeled as part of the gradual increasing resistance group. For the antibiotics that agree with these resistance groups, it may be possible to predict the trend of resistance by comparing the resistance rate and sale in the three countries.

Our study covered a 3-year period, which should be sufficient to demonstrate a possible resistance pattern in *E. coli* isolates found in community-acquired UTIs. While this was a time-limited study, our finding of a relatively high resistance rate in community-acquired *E. coli* UTIs to some antibacterial classes emphasizes the importance of monitoring the susceptibility of *E. coli* isolates, especially sulfonamides. Taken together with the correlation between antibacterial sales and resistance in the Faroe Islands, Iceland, and Denmark, these findings justify a reconsideration of antimicrobial strategies against the *E. coli* isolates in the Faroe Islands.

Authors' Contributions

M.D.M. PhD student, planned the study, collected data, analyzed data, and wrote the manuscript. K.G.K., PhD supervisor, planned the study, analyzed data, and wrote the manuscript. S.G. supervised the work and wrote the manuscript. H.G. planned and did the statistics together with M.D.M. and he wrote the manuscript. All authors read and approved the final manuscript.

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Disclosure Statement

No competing financial interests exist.

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Paper IV

Marita Debess Magnussen, Helga Erlendsdóttir, Shahin Gaini, Thorolfur Gudnason and Karl G. Kristinsson. *Streptococcus pneumoniae* - Antimicrobial Resistance and Serotypes of Strains Carried by Children and Causing Invasive Disease in the Faroe Islands. *Microb Drug Resist,* Published online: 19 Jun 2018.

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Streptococcus pneumoniae: Antimicrobial Resistance and Serotypes of Strains Carried by Children and Causing Invasive Disease in the Faroe Islands

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Knowledge about pneumococcal carriage, antibacterial resistance, serotype prevalence, and prevalence of invasive pneumococcal disease (IPD) after introduction of pneumococcal conjugate vaccines (PCVs) is lacking in the Faroe Islands. PCV-7 was introduced in 2008 and PCV-13 in 2010. The aim was to obtain knowledge on serotypes and antimicrobial resistance in pneumococci from carriage in children attending day-care centers (DCCs) and invasive isolates. Nasopharyngeal swabs were collected from 607 healthy children attending DCCs in the Faroe Islands in January to March in 2009, 2010, and 2011. Pneumococci were cultured selectively, tested for antibacterial susceptibility, and serotyped. Data from IPD isolates from 1974 to 2016 from the Department of Microbiology, National Hospital of the Faroe Islands, and typed and stored at Staten Serum Institute were also analyzed. Of the 607 screened children, 45% were pneumococcal carriers, 50% in 2009, 40% in 2010, and 42% in 2011. Antibiotic resistance in pneumococci was rare both in carriers and patients. Five penicillin nonsusceptible pneumococci were found in carriers (1.8%) and one among the invasive isolates (1.7%). The most common serotypes in carriage were 6B and 6A in 2009, serotype 3 and 6C in 2010, and serotype 11 and 6C in 2011. Serotype 6B was not found in 2011. The most common serotypes among IPD were 7F and 3. Pneumococcal carriage prevalence in healthy children attending DCCs in the Faroe Islands was low and antibacterial resistance was rare, compared with Iceland. The results suggest a possible serotype shift, reduction in antibacterial use, and PCV-7/13 serotype decrease in IPD after the introduction of pneumococcal vaccinations in children.

Keywords: *Streptococcus pneumoniae*, serotypes, carriage, resistance

Introduction

Streptococcus pneumoniae (pneumococcus) is a significant cause of bacterial infections worldwide, ranging from common infections such as acute otitis media and pneumonia to life-threatening invasive infections such as sepsis and meningitis.[1] Despite the ability to cause life-threatening disease, pneumococci are also carried asymptomatically in the nasopharynx, especially in children.[2,3]

Day-care centers (DCCs) provide a favorable milieu for pneumococcal spread, where children with an immature immune system and poor hygiene practices are crowded together.[4] Therefore, children attending DCCs are more likely to be pneumococcal carriers than children not attending DCCs.[5–7] Common infections such as respiratory tract infections are frequent causes for prescribing antibiotics and thus contribute to a high consumption of antibiotics in the community.[8] Several studies have shown an association between high antimicrobial use and increasing antimicrobial resistance in pneumococci.[9–11] Penicillin has been the drug of choice for treatment of pneumococcal infections in Scandinavian countries for decades. In the mid-1960s, the first strain of penicillin nonsusceptible pneumococci (PNSP) was reported[12] and during the last decades, antimicrobial resistance of pneumococci has increased in many countries, including Iceland.[13,14]

Knowledge about carriage rates, antibacterial resistance, and serotype prevalence after introduction of pneumococcal

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conjugate vaccines (PCVs) is lacking in the Faroe Islands. Pneumococcal vaccination was introduced with the 7-valent vaccine in 2008 and the 13-valent vaccine in 2010. The Faroe Islands are relatively isolated small populated islands and suitable for carriage, antibacterial resistance, and pneumococcal serotype studies. Iceland is a neighboring Nordic country with cultural similarities. In Iceland, there have been several studies on pneumococcal carriage, resistance, and serotype prevalence before and after the introduction of PCVs.

The objective of this Faroe Islands study were to describe pneumococcal carriage in healthy children aged 0 to 7 years from 2009 to 2011, to describe antibacterial resistance and serotype prevalence in these carriage isolates and invasive pneumococcal disease (IPD) isolates from 1974 to 2016, and to compare the results with similar studies from Iceland.

Materials and Methods

Study population

In 2009, 2010, and 2011 (January to March), nasopharyngeal swabs were collected from healthy children attending DCCs in the Faroe Islands. The Faroe Islands is a self-governing country under the sovereignty of Denmark with a population passing 50,000 inhabitants in 2017. Children aged ≤7 years represented about 11% of the population of the Faroe Islands.[15] In January 2012, 57 DCCs were registered in the Faroe Islands[16] and 30 DCCs were selected as study sampling sites (11 in 2009, 12 in 2010, and 7 in 2009). The DCCs were selected in different geographic locations to be representative for the Faroe Islands. The target population was ∼1000 children, of which a total of 607 with an age range from 0 to 7 years were included in the study.

Questionnaire

The parents answered a questionnaire on the use of antibiotics, vaccinations, number of siblings younger than the age of 6 years, and the occurrence of otitis media in their child. The questionnaires were returned from all the participants (100%).

Nasopharyngeal swabs

On a selected study day, two research nurses visited the DCCs after an informed consent from the parents or the legal guardians of the children had been obtained. Each child was sampled only once. The samples were obtained with a sterile medical applicator, Copan® (Transport medium swabs, Copan, Italy).

Laboratory procedures

The samples were stored for a maximum of 24 hr at 2–8°C, before they were inoculated on blood agar containing gentamicin (5 mg/L). An optochin disc (Oxoid, UK) was placed in the center and the agar plates were incubated anaerobically at 35°C for 18–24 hr. α-hemolytic colonies exhibiting morphology suggestive of *S. pneumoniae* were picked. Identification was obtained by susceptibility to optochin.

Antibacterial susceptibility testing

All pneumococcal isolates were tested for antimicrobial susceptibilities to erythromycin, clindamycin, tetracycline, chloramphenicol, and trimethoprim/sulfamethoxazole, using disc diffusion and the EUCAST methods and criteria (www.eucast.org). The isolates were screened for penicillin non-susceptibility with oxacillin discs and penicillin minimum inhibitory concentration (MIC) measured for all oxacillin-resistant isolates using the *E* test (BioMérieux, France). Isolates with MIC ≤0.064 mg/L to penicillin were considered fully susceptible to penicillin and isolates with >0.064 mg/L were defined as PNSP.

Serotyping

Serotyping was performed by Pneumococcal Latex antisera from Statens Serum Institute, (SSI, Denmark).[17]

Invasive isolates

The laboratory at the National Hospital of the Faroe Islands has a collaboration with Staten Serum Institute regarding registering and storing of bacterial isolates. Information about age, sex, susceptibility testing, serotype, and origin of the IPD isolates from 1974 to 2016 was obtained from the SSI. There were 60 pneumococcal isolates from blood and spinal fluids. Before 1978, all microbiological samples were sent to SSI for routine diagnostics. A local laboratory of clinical microbiology at the National Hospital, Faroe Islands, was established in 1978. Blood cultures were done in the Faroe Islands from 1978 to 1999 using conventional blood culture bottles, but after 1999, using the BacTec blood culturing platform. The 60 IPD isolates probably do not represent all invasive infections from 1974 until 2007, so that the exact number of IPD cases before 2007 is not known. Since 2007, all invasive pneumococci have been sent to SSI as a part of the mandatory national surveillance of pneumococcal serotypes in the Kingdom of Denmark, including the Faroe Islands.

Statistical analyses

Univariate analysis was performed using chi-square test with df = 2 < 5.991 and z-score with p *value* ≤0.05 considered significant. MATLAB was used to perform the statistical analyses.

Ethical approval. Ethical approval for the study was obtained from the Scientific Ethical Board of the Faroe Islands on the 7th of November in 2008, and the data collection approval was obtained from the data Protection Agency on the 6th of June in 2008.

Results

Pneumococcal carriage and results of questionnaire

Of the 607 children sampled, 45% ($n = 271$) carried pneumococci, 50% (133 of 265), 40% (89 of 225), and 42% (49 of 117) in the years 2009, 2010, and 2011, respectively. The difference in carriage prevalence between the years was significant ($X^2 = 6.03$ with a critical region <5.991).

Carriage was higher in children younger than the age of 3 years ($p = < 0.001$). Pneumococcal carriers were significantly younger in 2009 (3.49 years) than in 2010 (4.24 years) and in 2011 (4.07 years) ($p = 0.0002$), and the carriage prevalence was higher in 2009 than in 2010 and 2011 (p value = 0.011) (Fig. 1).

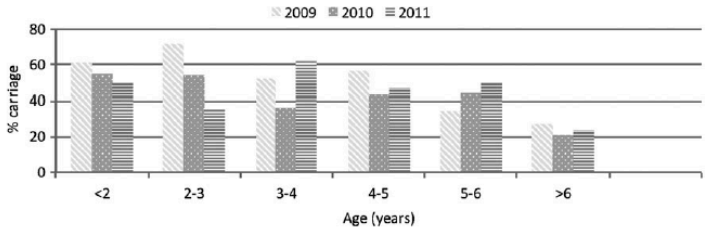

FIG. 1. Pneumococcal carriage rate according to age in children attending DCC in the Faroe Islands in 2009, 2010, and 2011. DCC, day-care center.

Pneumococcal carriers did not use antibiotics more often than nonpneumococcal carriers. However, pneumococcal carriers used antibiotics more often in 2009 ($n=43$, 32%) than pneumococcal carriers in 2010 and 2011($n=10$ and 24, 25%) ($p=0.017$). The proportion of children having had otitis media and having siblings younger than the age of 6 years was the same in pneumococcal carriers and noncarriers.

Antibacterial susceptibility, vaccination, and serotype distribution

Reduced susceptibility to one or more antimicrobial was found in 15 (11.3%), 8 (9.0%), and 7 (14.3%) pneumococcal isolates in 2009, 2010, and 2011, respectively (Table 1). Erythromycin resistance was found in four isolates (Table 1). These four isolates belonged to three different serotypes (19F ($n=2$) in 2009, 6C in 2010, and serogroup 11 in 2011). The erythromycin-resistant isolate of serotype 6C was also nonsusceptible to penicillin. Five PNSPs were found, three in 2009 (two serotype 19F and one nontypeable) and two in 2010 (serotype 6C and serogroup 23 [not serotype 23F]).

Among the 271 isolates, 27 different serotypes were identified. The two most frequent serotypes in each year were 6B and 6A in 2009, 3 and 6C in 2010, and serogroup 11 and 6C in 2011 (Fig. 2).

In 2008, Faroese children were vaccinated with PCV-7 and in spring 2010 PCV-7 was replaced with PCV-13. In 2010, 28% children had been PCV-7/13 vaccinated, nine (10%) of which carried pneumococci. In 2011, 65% children had been vaccinated, of which 18 (37%) carried pneumococci. There was no statistical difference in vaccination status of carriers and noncarriers in 2010 and in 2011. In 2010, four fully vaccinated children carried vaccine serotypes, two with serotype 6B and two with serotype 19F, and one child vaccinated with two doses carried serotype 19A. Nine children were carriers of serotype 3 in 2010, none was vaccinated. In 2011, one child vaccinated with one dose carried serotype 23F, and one child fully vaccinated carried serotype 3. Twelve children, four of whom were fully vaccinated, were carriers of serotype 11 (Fig. 2). PCV-7 serotypes accounted for 58, 29, and 5, and additionally, PCV-13 for 32, 23, and 3 isolates in the years 2009, 2010, and 2011, respectively.

Invasive isolates—antimicrobial susceptibility and serotypes

Of the 60 invasive isolates, only one was PNSP, serotype 23B (penicillin MIC=0.25) found in 2016. Three isolates were erythromycin resistant, one of serotype 15A in 2002, also multiresistant, and two isolates of serotype 14 in 2005 and 2006. The most frequent serotypes among invasive isolates were serotypes 3 ($n=8$) and 7F ($n=7$) (Table 2). Serotypes 7F and 3 were the most frequent serotypes in patients older than 7 years of age. Nine isolates of serotype 3 were recorded (in 2005, 2010, 2011, 2013, 2014, and 2016). Seven

TABLE 1. ANTIMICROBIAL SUSCEPTIBILITIES OF *STREPTOCOCCUS PNEUMONIAE* ISOLATES FROM CHILDREN ATTENDING DAY-CARE CENTERS IN THE FAROE ISLANDS IN 2009, 2010, AND 2011

	2009 n=*133* *R/I (%)*	*2010* n=*89* *R/I (%)*	*2011* n=*49* *R/I (%)*	*2009/2010/2011* n=*271* *ave R/I%*	*Penicillin G MIC (mg/L)*
Penicillins					
Oxacillin	8 (6.0)	6 (6.7)	1 (1.1)	5.5	
Penicillin G	3 (2.3)	2 (2.2)	0 (0)	1.7	0.125 to 0.25
Erythromycin	2 (1.5)	1 (1.1)	1 (1.1)	1.1	
Chloramphenicol	0 (0)	0 (0)	0 (0)	0	
Clindamycin	2 (1.5)	1 (1.1)	0 (0)	1.0	
Tetracycline	3 (2.3)	1 (1.1)	4 (4.5)	1.8	
Sulfonamides/trimethoprim	5 (3.8)	3 (3.4)	2 (2.2)	2.9	

Resistance is listed as (R/I) and penicillin is measured in MIC.
MIC, minimum inhibitory concentrations; (R/I), resistant/intermediate.

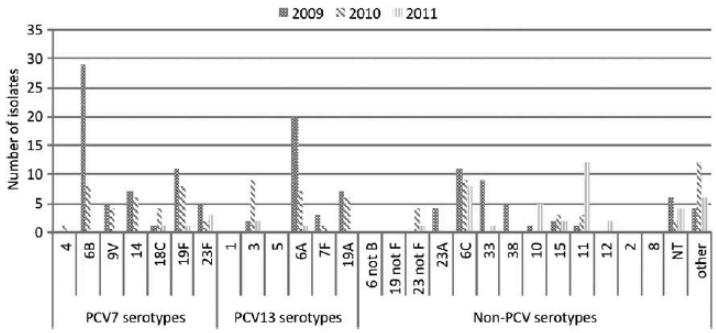

FIG. 2. *Streptococcus pneumoniae* serotypes from children attending DCC in the Faroe Islands in 2009, 2010, and 2011.

invasive isolates were from children. One 2-year-old child had the PCV-13 serotype 7F in 2010. The isolate was recovered from spinal fluid (Table 2). There was a possible decrease in PCV-7/13 serotypes among IPD from the period 2002 to 2016. From 2002 to 2006, there were 11 PCV-7/13 serotypes and 5 non-vaccine types (NVT). From 2007 to 2011, there were 21 PCV-7/13 and 1 NVT, and from 2012 to 2016, there were 6 PCV-7/13 and 9 NVT (Fig. 3).

Discussion

The prevalence of PNSP was low in the Faroe Islands compared with Iceland and Denmark,[7,10,18–20] and this may be because of conservative use of antibiotics in the Faroese community. Our results suggest reduced antibacterial use after vaccine introduction. Antibacterial susceptibility among pneumococci in the Faroese community is similar as in Denmark, Sweden, and Norway.[21–24] Another possible explanation may be that the macrolide of choice in the Faroe Islands is a short-acting erythromycin, whereas the long-acting azithromycin is mainly used in Iceland.[25] Azithromycin may have been a contributing factor to the circulation and increase of 19F clone in Iceland, which is often associated with PNSP among pneumococcal carriers.[19] The pneumococcal carriage rate in the Faroe Islands may also be a factor, for its prevalence is lower in the Faroe Islands than in Iceland and Denmark.[20,26] Furthermore, the PNSP pneumococcal clones may not have the same opportunity to circulate in the Faroe Island because of its small and rural population.

A significant difference in pneumococcal carriage in the Faroe Islands was observed between the years 2009, 2010, and 2011, with the highest pneumococcal carriage in 2009. The most likely reason for the difference could be that the children were significantly younger in 2009 than in 2010 and 2011, and in our study, children younger than the age of 3 years were significantly more often pneumococcal carriers than older children, this is consistent with other studies.[20,22]

The most common serotypes among children attending DCCs were 6B and 6A in 2009, serotype 3 and 6C in 2010, and serotype 11 and 6C in 2011. Serotype 6B was not found in 2011. There appeared to be a serotype shift with a reduction in PCV-13 serotype carriage from 2010 to 2011, since 9 vaccinated children carried five PCV-13 serotypes in 2010, and 18 vaccinated children carried three PCV-13 serotypes in 2011.

Nine healthy children carried serotype 3 in 2010, none of them had been vaccinated, and the most common serotypes in patients were 3 and 7F, however, no children had an invasive pneumococcal infection caused by serotype 3. PCV-13 vaccination program in Denmark has not lead to a reduction of the incidence of IPD caused by serotype 3.[27]

TABLE 2. SEROTYPES OF INVASIVE *STREPTOCOCCUS PNEUMONIAE* FROM THE FAROE ISLANDS FROM 1974 TO 2016

Year	*Serotype*
1974	34
1975	14, 7F, 8
1985	12F
1995	45
1997	-
2002	19F, **15A**
2004	33F, 14, 4, 7F
2005	4, 3, 20 (*n* = 2), 18C, **14**, 6B, 6A
2006	**14**, 22F
2007	7F, 1 (*n* = 3), 9 V, 19F
2008	19F, 1
2009	7F (*n* = 2), 9 V, 6B, 33F
2010	7F, 3, 12F, 19A, 11A
2011	3 (*n* = 2), 7F, 1, 23F
2012	23A
2013	22F (*n* = 2), 3 (*n* = 2), 35F, 15B
2014	7F, 3 (*n* = 2), 15C
2015	21
2016	3, 15C, **23B**

Serotypes marked with bold are resistant to antimicrobials and serotypes underlined belong to PCV-7/13. PCV, pneumococcal conjugate vaccine.

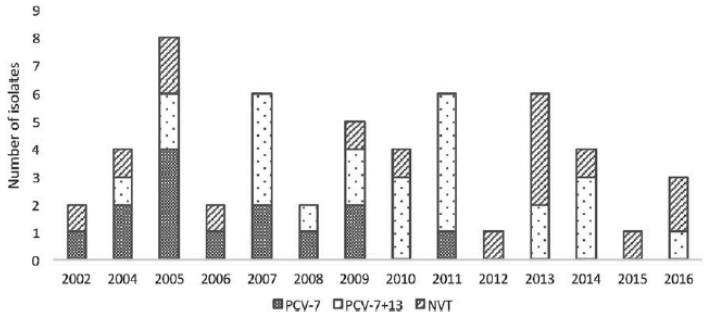

FIG. 3. Serotype distribution in invasive isolates in the Faroese community, 2002–2016. PCV-7; serotypes 4, 6B, 9V, 14, 18C, 19F, 23F, PCV-13 serotypes in PCV-7 plus serotypes 1, 3, 5, 6A, 7F, 19A, and NVT; other than in PCV-10 and PCV-13. NVT, non-vaccine types; PCV, pneumococcal conjugate vaccine.

Our results suggest that this could also be the case in the Faroe Islands. Serotype 7F is considered to have high invasive potential,[28] and therefore its frequency among IPD in the Faroese community was not unexpected.

The results of the historical IPD isolates serotyped at SSI should be interpreted cautiously because of the limited information about sample processing and laboratory methods used. The Faroe Islands did not have an IPD surveillance before 2007, and therefore, there could have been more IPD cases than the 60 recorded. Iceland and Denmark reported higher number of invasive pneumococci than the Faroe Islands,[29] and the small numbers of isolates from the Faroe Islands may therefore not give a true representation of the Faroese situation.

In conclusion, pneumococcal carriage rates in children attending DCCs were lower than in the neighboring countries, and antimicrobial resistance in pneumococci was extremely rare in the Faroe Islands. Pneumococcal vaccination has probably reduced the incidence of PCV-7/13 serotypes among IPD cases, and there is indication of serotype shift in pneumococcal carriage in DCCs. Further monitoring is important to detect new serotypes in invasive disease and changes in disease prevalence.

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Disclosure Statement

No competing financial interests exist.

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Appendix I

Table 7. Characteristics of pneumococcal carriers (*n* = 271) and non-carriers (*n* = 336) from healthy children attending DCCs in the Faroe Islands in 2009, 2010 and 2011.

	Year of surveillance			
Parameter (%)	**2009**	**2010**	**2011**	**Significance**
Carriers	*n* = 133	*n* = 89	*n* = 49	
Male	71 (26.8)	52 (23.1)	22 (18.8)	
Female	62 (23.4)	37 (16.4)	27 (23.1)	$X^2 = 1.38$
Non-carriers	*n = 132*	*n* = 136	*n* = 68	
Male	79 (29.8)	60 (26.7)	26 (22.2)	
Female	53 (20.0)	76 (33.8)	42 (35.9)	
Antibiotic use in carriers	43	10	24	*p* = 0,017
At sampling day	2	0	4	
Last month	9	1	7	
6 months before	32	9	13	
Antibiotic use in non-carriers	41	23	20	*p* = 0.947
At sampling day	6	2	3	
Last month	11	4	9	
6 months before	24	17	8	
OM in carrier	56	36	20	*p* = 0.242
1	20	8	9	
2-3	16	8	3	
3-4	5	6	1	
>4	15	14	7	
OM in non-carrier	38	62	31	*p* = 0.862
1	14	12	8	
2-3	9	16	7	
3-4	8	13	2	
>4	21	21	14	

Vaccination in carriers	0	9	18	$p_{2010} = 0.729$
0	133	80	31	
1	0	1	2	
2	0	2	1	
3	0	6	15	
Vaccination in non-carriers	0	16	14	$p_{2011} = 0.247$
0	132	120	54	
1	0	0	0	
2	0	3	1	
3	0	13	13	
Carrier siblings <6 yrs old				
Yes	68	48	26	
Non-carrier siblings <6 yrs old				
Yes	54	63	46	$p = 0.191$

Appendix II

Study II-..... Pamphlet about the research in GAS

Study III-.....Pamphlet about the research in *E. coli*

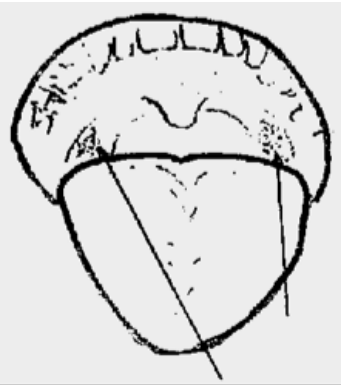
Study II and III- .....Informed consent

Study II- Pamphlet about the research in GAS

Faldari til sjúklingar

Antibiotika-kanning fyri *Streptococcus pyogenes* (strep A)

Gransking í mótstøðuførum bakterium



Í september 2008 fór ein ph.d.-verkætlan í gongd, sum er fíggjað av Granskingarráðnum. Í hesari verkætlanini nýtist mær hjálp tykkara. Verkætlanin fer at snúgva seg um antibiotika-mótstøðuførið hjá bakterium, og er hetta ongantíð kannað í Føroyum áður.

Les meira í hesum faldaranum og á heimasíðuni www.hfs.fo.

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Antibiotika-kanning

Gransking í mótstøðuførum bakterium

Góðu luttakarar

Eg eiti Marita Debess Magnussen og eri útbúgvin medisinskur mikrobiologur. Í september 2008 fór eg í gongd við eina ph.d.-verkætlan, sum er fíggjað av Granskingarráðnum, BP Amoco og Chevron Texaco. Í hesari verkætlanini nýtist mær hjálp tykkara. Verkætlanin fer at snúgva seg um antibiotika-mótstøðuførið hjá bakterium, og er hetta ongantíð kannað í Føroyum áður.

Í tí føroyska samfelagnum er sera lítil vitan um antibiotika-mótstøðuførið hjá bakterium. Antibiotika-mótstøðuførar bakteriur eru í vøkstri runt allan heimin. Hetta hevur havt við sær, at sjúklingar ikki kunnu viðgerast við antibiotika ímóti hesum bakterium og tí mugu ígjøgnum eina drúgvari viðgerð.

Í Føroyum finnast eingin tøl fyri hesum, men sammeta vit okkum við grannalondini, so er væl hugsandi, at sama er galdandi í Føroyum. Í Íslandi til dømis, har nógv breiðsporað antibiotika

hevur verið nýtt, er trupulleikin viðvíkjandi mótstøðuføri sera umfatandi.

Henda granskingarverkætlanin er av stórum týdningi fyri tað føroyska samfelagið, tí hon fer at geva okkum týdningarmikla vitan um støðuna í antibiotika-mótstøðuførinum í humanum bakterium. Eingin dáta eru skrásett í Føroyum um mótstøðuførið hjá bakterium ímóti antibiotika, og tískil er eingin vitan, hvørt tann medisinska viðgerðin í løtuni er munagóð.

Tá ið vit hava fingið vitan um antibiotika-mótstøðuførið í humanum bakterium, so kunnu teir medisinsku myndugleikarnir nýta hesa vitan, tá ið sjúkur, sum eru komnar av hesum bakterium, skulu viðgerast. Eitt nú kunnu fleiri útgreiningar av sjúkuavgerðum gerast, og somuleiðis kann smalsporað antibiotika verða nýtt.

Prof. Karl K. Kristinson er vegleiðari í verkætlanini, og Pál Weihe, yvirlækni, er kliniskur ábyrgdari og hevur ábyrgdina av tí medisinska økinum. Eg havi valt at kanna mótstøðuførið í trimum vanligum bakterium, ið fólk ofta eru berarar av og tískil eisini ofta verða viðgjørd fyri.

Eg havi avgjørt at taka royndir av sjúklingum, sum koma til viðtalu hjá kommunulæknum orsakað av hálspínu, tí tað er

bakterian *Streptococcus pyogenes*, vanliga nevnd strep. A sum oftani er atvoldin til hálsbruna.

Til hesa kanningina nýtist mær eina hálsroynd frá tygum. Kommunulæknin tekur eina eyka roynd til mín, og tygum kenna onga pínu av hesum. Síðan verður royndin dyrkað fyri at vita, um tygum hava *Streptococcus pyogenes*. Kommunulæknin fær svar upp á royndina 2 dagar eftir, at hon er tikin, og tann medisinska viðgerðin hjá tygum er ikki tengd at dyrkingunum. Henda royndin er talmerkt, so tað er bert kommunulæknin, sum veit, hvør tygum eru.

Antibiotika-mótstøðuførið hjá bakteriuni verður síðan kannað og sammett við mótstøðuførið í Danmark og Íslandi.

Tað er sjálvboðið at luttaka í verkætlanini. Um tygum velja ikki at luttaka, so fær hetta ongar avleiðingar fyri tygum. Allar upplýsingar, sum vit fáa frá luttakarunum, verða skrásettar í trúnaði. Tað verða bert undirritaða, Pál Weihe og kommunulæknin, ið fara at síggja tað, sum verður skrásett, og er henda skráseting góðkend av Dátueftirlitinum.

Tygum eru vælkomin at venda tygum til mín, um tygum hava nakrar spurningar hesum viðvíkjandi.

Study III - Pamphlet about the research in *E. coli*

Faldari til sjúklingar

Antibiotika-kanning fyri *Escherichia coli* (*E.coli*)

Gransking í mótstøðuførum bakterium

Í september 2008 fór ein ph.d.-verkætlan í gongd, sum er fíggjað av Granskingarráðnum. Í hesari verkætlanini nýtist mær hjálp tykkara. Verkætlanin fer at snúgva seg um antibiotika-mótstøðuførið hjá bakterium, og er hetta ongantíð kannað í Føroyum áður.

Les meira í hesum faldaranum og á heimasíðuni www.hfs.fo.

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Antibiotika-kanning

Gransking í mótstøðuførum bakterium

Góðu luttakarar

Eg eiti Marita Debess Magnussen og eri útbúgvin medisinskur mikrobiologur. Í september 2008 fór eg í gongd við eina ph.d.-verkætlan, sum er fíggjað av Granskingarráðnum, BP Amoco og Chevron Texaco. Í hesari verkætlanini nýtist mær hjálp tykkara. Verkætlanin fer at snúgva seg um antibiotika-mótstøðuførið í bakterium, og er hetta ongantíð kannað í Føroyum áður.

Í tí føroyska samfelagnum er sera lítil vitan um antibiotika-mótstøðuførið hjá bakterium. Antibiotika-mótstøðuførar bakteriur eru í vøkstri runt allan heimin. Hetta hevur havt við sær, at sjúklingar ikki kunnu viðgerast við antibiotika ímóti hesum bakterium og tí mugu ígjøgnum eina drúgvari viðgerð.

Í Føroyum finnast eingin tøl fyri hesum, men sammeta vit okkum við grannalondini, so er væl hugsandi, at sama er galdandi í Føroyum. Í Íslandi til dømis, har nógv breiðsporað antibiotika hevur verið nýtt, er trupulleikin viðvíkjandi mótstøðuføri sera umfatandi.

Henda granskingarverkætlanin er av stórum týdningi fyri tað føroyska samfelagið, tí hon fer at geva okkum týdningarmikla vitan um støðuna í antibiotika-mótstøðuførinum í humanum bakterium. Eingin dáta eru skrásett í Føroyum um mótstøðuførið hjá bakterium ímóti antibiotika, og tískil er eingin vitan, hvørt tann medisinska viðgerðin í løtuni er munagóð.

Tá ið vit hava fingið vitan um antibiotika-mótstøðuførið í humanum bakterium, so kunnu teir medisinsku myndugleikarnir nýta hesa vitan, tá ið sjúkur, sum eru komnar av hesum bakterium, skulu viðgerast. Eitt nú kunnu fleiri útgreiningar av sjúkuavgerðum gerast, og somuleiðis kann smalsporað antibiotika verða nýtt.

Prof. Karl K. Kristinson er vegleiðari í verkætlanini, og Pál Weihe, yvirlækni, er kliniskur ábyrgdari og hevur ábyrgdina av tí medisinska økinum. Eg havi avgjørt at kanna mótstøðuførið í trimum vanligum bakterium, ið fólk ofta eru berarar av og tískil eisini ofta verða viðgjørd fyri.

Eg havi valt at taka royndir av sjúklingum, sum koma til viðtalu hjá kommunulæknum orsakað av sjúkueyðkennum upp á landrásbruna. Tað er bakterian *Escherichia coli*, vanliga nevnd (E.coli), sum oftani er atvoldin til landrábruna.

Til kanningina nýtist mær eina landroynd frá tygum. Kommunulæknin tekur eina eyka roynd til mín. Síðan verður royndin dyrkað fyri at vita, um bakterian *Escherichia coli* er í royndini. Kommunulæknin fær svar upp á royndina 2 dagar eftir, at hon er tikin, og tann medisinska viðgerðin hjá tygum er ikki tengd at dyrkingunum. Henda royndin er talmerkt, so tað er bert kommunulæknin, sum veit, hvør tygum eru.

Antibiotika-mótstøðuførið hjá bakteriuni verður síðan kannað og sammett við mótstøðuførið í Danmark og Íslandi.

Tað er sjálvboðið at luttaka í verkætlanini. Um tygum velja ikki at luttaka, so fær hetta ongar avleiðingar fyri tygum. Allar upplýsingar, sum vit fáa frá luttakarunum, verða skrásettar í trúnaði. Tað verða bert undirritaða, Pál Weihe og kommunulæknin, ið fara at síggja tað, sum verður skrásett, og er henda skráseting góðkend av Dátueftirlitinum.

Tygum eru vælkomin at venda tygum til mín, um tygum hava nakrar spurningar hesum viðvíkjandi.

Study II and III - Informed consent

Samtykki til sjúklingar
Antibiotika kanning

Gransking í mótstøðuførum bacterium

Hervið játti eg, at eg hava móttikið kunning um verkætlanina og eg játti at vera við í granskingarverkætlanini hjá Maritu Debess Magnussen

Hervið játti eg, at eg havi fingið kunning um at luttøkan í verkætlanini er sjálvboðin og at tað ber til at hálsa um og taka seg burtur úr kanningini uttan at tað fær avleiðingar fyri meg.

Navn

_____

Føðingardagur

_____

Bústaður

_____

_____

Dagfesting og undirskrift

Dato_____

_____

Appendix III

Study IV-.....Pamphlet about the research in pneumococci

Study IV-.....Informed consent

Study IV-.....Questionnaire

Study IV- Pamphlet about the research in pneumococci

Kunningarskriv til foreldur

Antibiotika-kanning fyri *Streptococcus pneumoniae*

Gransking í mótstøðuførum bacterium

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Antibiotika-kanning

Gransking í mótstøðuførum bakterium

Góðu foreldur

Eg eiti Marita Debess Magnussen og eri útbúgvin medisinskur mikrobiologur. Í september 2008 fór eg í gongd við eina ph.d.-verkætlan, sum er fíggjað av Granskingarráðnum, BP Amoco og Chevron Texaco. Í hesari verkætlanini nýtist mær hjálp tykkara. Verkætlanin snýr seg um antibiotika-mótstøðuførið hjá bakterium, og er hetta ongantíð kannað í Føroyum áður.

Í tí føroyska samfelagnum er lítil vitan um antibiotika-mótstøðuførið hjá bakterium. Antibiotika-mótstøðuførar bakteriur eru í vøkstri runt allan heimin. Hetta hevur havt við sær, at sjúklingar ikki kunnu viðgerast við antibiotika ímóti sjúkum, sum koma av hesum bakterium, og tí mugu ígjøgnum eina drúgvari viðgerð.

Í Føroyum finnast eingin tøl fyri hesum, men sammeta vit okkum við grannalondini, so er væl hugsandi, at sama er galdandi í Føroyum. Í Íslandi til dømis, har nógv breiðsporað antibiotika

hevur verið nýtt, er trupulleikin viðvíkjandi mótstøðuføri sera umfatandi.

Henda granskingarverkætlanin er av stórum týdningi fyri tað føroyska samfelagið, tí hon fer at geva okkum týdningarmikla vitan um støðuna í antibiotika-mótstøðuførinum í humanum bakterium. Eingin dáta eru skrásett í Føroyum um mótstøðuførið hjá bakterium ímóti antibiotika, og tískil er eingin vitan, hvørt tann medisinska viðgerðin í løtuni er munagóð.

Tá ið vit hava fingið vitan um antibiotika-mótstøðuførið í humanum bakterium, so kunnu teir medisinsku myndugleikarnir nýta hesa vitan, tá ið sjúkur, sum eru komnar av hesum bakterium, skulu viðgerast. Eitt nú kunnu fleiri útgreiningar av sjúkuavgerðum gerast, og somuleiðis kann smalsporað antibiotika verða nýtt.

Prof. Karl K. Kristinson er vegleiðari í verkætlanini, og Pál Weihe, yvirlækni, er kliniskur ábyrgdari og hevur ábyrgdina av tí medisinska økinum. Eg havi avgjørt at kanna mótstøðuførið í trimum vanligum bakterium, ið fólk ofta eru berarar av og tískil eisini ofta verða viðgjørd fyri.

Børn eru berarar av bakteriuni *Streptococcus pneumoniae*, uttan at henda bakterian er til vanda fyri barnið. Tí havi eg avgjørt

at taka royndir av børnum, tá ið eg skal kanna antibiotika-mótstøðuførið hjá *Streptococcus pneumoniae*.

Til kanningina nýtist mær nasaroyndir frá barni/børnum tygara. Tveir sjúkrarøktarfrøðingar koma út á barnagarðin/vøggustovuna, har børnini ganga, at taka eina roynd úr nøsini hjá barninum við einum vattpinni. Barnið verður bert potað eina ferð og fær eina frágreiðing um tað, sum fer fram. Barnið kann kenna ein sting av hesum, men fer onga pínu at kenna av royndunum.

Meðan kanningin fer fram, verður antin eitt foreldur ella ein annar persónur til staðar, sum barnið kennir seg tryggan hjá. Síðan dyrka vit royndina fyri at vita, um barnið er berari av *Streptococcus pneumoniae*. Royndin verður dyrkað av mær á Heilsufrøðiligu starvsstovuni. Tygum fáa ikki at vita um barnið er berari av *Streptococcus pneumoniae,* men hava tygum nakað at spyrja um, so eru tygum vælkomin at ringja til mín.

Verður nakað funnið, sum krevur medisinska viðgerð, setur Pál Weihe seg í samband við foreldur/verja.

Antibiotika-mótstøðuførið hjá bakteriunum verður kannað og sammett við mótstøðuførið í Danmark og Íslandi.

Allar innsavnaðar upplýsingar verða skrásettar í trúnaði. Tað verða bert undirritaða og Pál Weihe, ið fara at síggja tað, sum

verður skrásett, og henda skráseting er góðkend av Dátueftirlitinum.

Um tygum játta, at lata barn tygara luttaka í verkætlanini, so kunnu tygum geva dagstovninum tann leysa lepan innan **05. januar 2011**. Tað er sjálvboðið at luttaka í verkætlanini, og um tygum ikki ynskja at luttaka, fær hetta ongar avleiðingar fyri tygum. Barnið kann broyta meining og taka seg burturúr kanningini, uttan at tað fær avleiðingar fyri barnið. Um játtan fæst frá tygum, fáa tygum eitt bræv við nærri kunning viðvíkjandi tíðaráseting.

Tygum eru vælkomin at venda tygum til mín, um tygum hava nakrar spurningar hesum viðvíkjandi.

Study IV- Informed consent

Samtykki til foreldur
Antibiotika kanning

Hervið játti eg/vit, at vit hava móttikið kunning um verkætlanina og eg/vit játta at barn mítt/okkara kann vera við í granskingarverkætlanini hjá Maritu Debess Magnussen

Hervið játta eg/vit, at vit eru kunnaði um at luttøkan í verkætlanini er sjálvboðin og at tað ber til at hálsa um og taka seg burtur úr kanningini uttan at tað fær avleiðingar fyri barnið.

Barnið eitur

_____

Føðingardagur

_____

Navnið ella nøvnini hjá foreldrum/verja

_____

_____

Bústaður hjá barninum

_____

_____

Telefon

_____

Dagfesting og undirskrift foreldranna/verja

Dato_____

_____

Dato_____

_____

Dagfesting og undirskrift hjá barninum *

Dato_____

_____

* Um barnið dugir at skriva, so kann barnið skriva undir.

Study IV- Questionnaire

Antibiotika-kanning fyri *Streptococcus pneumoniae*

Gransking í mótstøðuførum bakterium

Góðu foreldur

Við hesum verður takkað fyri, at tygum loyva barn tygara at luttaka í hesari verkætlan.

Til kunning tygara kann upplýsast, at tveir sjúkrarøktarfrøðingar vera á dagstovninum, har barn tygara gongur ..... Tær fara at taka eina roynd úr nøsini hjá barni tygara. Tygum/tit eru vælkomin at vera til staðar. Fyrivarni skal takast fyri, at tygum/tit kunnu koma at bíða eina løtu.

Tygum verða biðin um at svara niðanfyri standandi spurningum. Send tað við postinum ella via meyl til: mdm@hfs.fo

Heilsufrøðiliga starvsstovan
Att: Marita D. Magnussen
V.U. Hammershaimbsgøta 11
FO-100 Tórshavn

Tekur barnið antibiotika í løtuni? ja____ nei____ veit ikki____

Um ja, hvat slag? _____

Hevur barnið fingið antibiotika tann síðsta mánaðin?

ja____ nei____ veit ikki____

Um ja, fyri hvat? _____

Hevur barnið fingið antibiotika innan tað síðsta ½ árið?

ja____ nei____ veit ikki____

Hevur barnið havt miðoyrnabruna?

Ja____ nei____ veit ikki____

Um ja, hvussu oftani?

_____

Er barnið koppsett ímót pneumokokkum?

ja___ nei___ veit ikki____

Um ja, hvussu oftani?

_____

Eru tað fleiri børn undir 6 ár í familjuni?

ja____ nei____

Dagfesting og
undirskrift_____