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Lotte M. Lambertsen, Zitta B. Harboe, Helle B. Konradsen, Jens Jørgen Christensen, Anette M. Hammerum. Non-invasive erythromycin-resistant pneumococcal isolates are more often non-susceptible to more antimicrobial agents than invasive isolates. *International Journal of Antimicrobial Agents*, 2009, 35 (1), pp.72. 10.1016/j.ijantimicag.2009.09.006 . hal-00556365

HAL Id: hal-00556365

<https://hal.science/hal-00556365>

Submitted on 16 Jan 2011

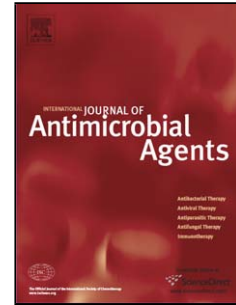
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PII: S0924-8579(09)00415-4
DOI: doi:10.1016/j.ijantimicag.2009.09.006
Reference: ANTAGE 3127

To appear in: *International Journal of Antimicrobial Agents*

Received date: 14-7-2009
Revised date: 27-8-2009
Accepted date: 2-9-2009

Please cite this article as: Lambertsen LM, Harboe ZB, Konradsen HB, Christensen JJ, Hammerum AM, Non-invasive erythromycin-resistant pneumococcal isolates are more often non-susceptible to more antimicrobial agents than invasive isolates, *International Journal of Antimicrobial Agents* (2008), doi:10.1016/j.ijantimicag.2009.09.006

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Non-invasive erythromycin-resistant pneumococcal isolates are more often non-susceptible to more antimicrobial agents than invasive isolates

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ARTICLE INFO

Article history:

Received 14 July 2009

Accepted 2 September 2009

Keywords:

Streptococcus pneumoniae

Serotype

Antibiotic resistance

Erythromycin

Penicillin

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ABSTRACT

Multiresistant *Streptococcus pneumoniae* infections are of great concern as treatment failures may occur with commonly used treatment regimens using β -lactams and macrolides. The proportion of non-susceptible *S. pneumoniae* differs from country to country. In Denmark, the proportion of invasive penicillin- or erythromycin-non-susceptible isolates is still low. The aim of this study was to characterise and compare invasive and non-invasive penicillin-non-susceptible and erythromycin-resistant pneumococcal isolates from the same geographic area and the same time period with respect to serotype and antibiotic susceptibility profile. We aimed to identify which serotypes were multiresistant among Danish isolates and to confirm or reject whether there was a difference in serotype distribution and resistance profiles between invasive and non-invasive isolates. We observed that non-invasive penicillin-non-susceptible pneumococci had higher serotype diversity than invasive isolates. This was not the case for erythromycin-resistant pneumococci. The dominant serotypes among non-susceptible invasive isolates were serotypes 9V and 14, whereas the dominant serotypes among non-susceptible non-invasive isolates were serotypes 19F, 14, 9V, 6B and non-typeable (NT). Non-invasive isolates were also more likely to be resistant to three or more antimicrobial agents than invasive isolates, however isolates being multiresistant were often co-resistant to the same antimicrobial agents.

1. Introduction

Infections caused by multiresistant *Streptococcus pneumoniae* are of great concern as they may cause treatment failures with common treatment regimens using β -lactams and macrolides. The proportion of pneumococci non-susceptible to antibiotics varies from country to country. Even in a relatively small geographic area such as Europe great differences have been reported. For example, in France 25–50% of all invasive isolates have been reported to be penicillin-non-susceptible pneumococci (PNSP) or erythromycin-resistant pneumococci (ERP), whereas in Norway only 1–5% of all invasive *S. pneumoniae* isolates were reported to be PNSP and 5–10% were ERP [1]. In Denmark, 3% of all invasive *S. pneumoniae* isolates were reported as PNSP and 6% were ERP in 2007 [2].

As a reference laboratory receiving pneumococcal isolates from all Danish departments of clinical microbiology, we have noticed that non-invasive PNSP and ERP isolates appear to be non-susceptible to more antimicrobial agents than invasive isolates. Only a few studies have looked at this previously. However, the observation may not be new, as it has been observed that a higher proportion of carriage isolates from healthy children were resistant compared with invasive isolates [3]. The aim of this study was to characterise and compare invasive and non-invasive PNSP and ERP isolates from the same geographic area and the same time period with respect to serotype and antibiotic susceptibility profile. We aimed to identify which serotypes were multiresistant among Danish isolates and to confirm or reject whether there was a difference in serotype distribution and resistance profiles between invasive and non-invasive isolates.

2. Material and methods

2.1. Bacterial isolates

From January 2006 to December 2007, all *S. pneumoniae* isolates from patients with invasive disease were received at Statens Serum Institut (Copenhagen, Denmark) from the 16 departments of clinical microbiology in Denmark. Non-invasive PNSP or ERP isolates from the same time period were received. In total, 1991 invasive isolates from hospitalised patients with meningitis, bacteraemia or other invasive disease (from other normal sterile site) and 308 non-invasive PNSP or ERP isolates from patients with various non-invasive diseases (from eye, ear, wound or lower/upper respiratory tract specimens) were received. One isolate per case was included in this study. Of the 1991 invasive pneumococcal isolates, 3.4% ($n = 68$) were PNSP, 6% ($n = 119$) were ERP and 0.8% ($n = 16$) were penicillin- and erythromycin-non-susceptible. Of the 308 non-invasive isolates, 97.7% ($n = 301$) were PNSP, 45.1% ($n = 139$) were ERP and 42.9% ($n = 132$) were penicillin- and erythromycin-non-susceptible isolates.

2.2. Antimicrobial susceptibility testing

The susceptibility of all isolates was investigated by determining the minimum inhibitory concentration of penicillin, erythromycin, ceftriaxone and ciprofloxacin by the Etest (AB BIODISK, Solna, Sweden) according to the manufacturer's guidelines. In addition, the isolates were tested for susceptibility to other antimicrobial agents [chloramphenicol, tetracycline and trimethoprim/sulfamethoxazole (SXT)] using Neo-

Sensitabs™ (Rosco Diagnostica, Taastrup, Denmark) on Danish Blood Agar plates. All plates were incubated in air plus 5% CO₂ at 36 °C for 22–24 h. Clinical and Laboratory Standards Institute (CLSI) breakpoints were used as recommended in 2007 [4]. *Streptococcus pneumoniae* ATCC 4696 was used as a quality control strain.

2.3. Serotyping

Isolates were serotyped using Pneumotest-Latex and capsular reaction test with type-specific pneumococcal antisera as recommended by the manufacturer (SSI Diagnostica, Hillerød, Denmark).

3. Results and discussion

Non-invasive PNSP had a higher diversity (with respect to serotype) than invasive PNSP isolates. This was not the case for ERP isolates. Invasive PNSP ($n = 68$) belonged to 15 different serotypes with serotype 9V (34%) and 14 (16%) being the dominant types, whereas invasive ERP ($n = 119$) belonged to 14 different serotypes with serotype 14 (70%) as the overall dominant type and 9V (8%) as the second most prevalent type (Fig. 1). The invasive penicillin- and erythromycin-non-susceptible isolates ($n = 16$) belonged to six different serotypes with no dominant type.

In contrast to the invasive isolates, the non-invasive PNSP isolates ($n = 301$) belonged to 22 different serotypes with 19F (18%), non-typeable (NT) (15%), 14

(12%) and 9V (8%) being the dominant types, and the non-invasive ERP isolates ($n = 139$) belonged to 14 different serotypes with the dominant serotypes being 19F (27%), NT (22%), 14 (15%) and 6B (9%). The non-invasive penicillin- and erythromycin-non-susceptible isolates ($n = 132$) belonged to 13 different serotypes also with the dominant serotypes NT (30%), 19F (26%), 14 (16%) and 6B (10%) (Fig. 1).

The dominant serotypes among invasive susceptible and non-susceptible isolates were partly overlapping. The most prevalent serotypes among all invasive isolates in 2006 and 2007 were serotypes 14 (12%), 1 (10%), 4 (8%), 7F (8%) and 9V (7%). Serotypes 14 and 9V were also dominant serotypes among non-susceptible isolates, thus very few serotype 1 and no serotypes 7F or 4 were observed among the non-susceptible isolates (invasive and non-invasive). One of the dominant serotypes among the non-invasive non-susceptible isolates (serotype 14) was also highly prevalent both among the non-susceptible and susceptible invasive isolates, whereas serotype 19F was a relatively prevalent serotype (4%) among invasive isolates. These observations are in agreement with previous studies reviewed by Henriques-Normark in 2007 [3] concluding that antibiotic resistance is more often found in serotypes prevalent among isolates from the nasopharynx of healthy carriers such as serotypes 9V, 6B, 14, 19F and 23F than in serotypes rarely found among carriage isolates such as serotypes 1 and 7F. Although the isolates analysed in this study were all from clinically ill patients, the serotypes of the non-invasive isolates from those patients corresponded to the serotypes observed for carriage isolates. The NT isolates were also highly prevalent among non-invasive non-susceptible isolates but

only represented by one isolate among invasive isolates in the study period. This was an expected result as NT isolates are known to be only very rarely invasive as they lack the required polysaccharide capsule [5–7].

Thirty-seven percent ($n = 25$) of the invasive PNSP isolates and 24% ($n = 73$) of the non-invasive PNSP isolates were only non-susceptible to penicillin and were susceptible to all other tested antimicrobial agents (Fig. 2). In contrast, 28% ($n = 19$) of the invasive PNSP and 49% ($n = 146$) of the non-invasive PNSP isolates were resistant to three or more of the tested antimicrobial agents (Fig. 2). Four non-invasive isolates were non-susceptible to at least six antimicrobial agents. Among the invasive PNSP isolates, 43% ($n = 29$) were co-resistant to SXT, 24% ($n = 16$) to erythromycin, 19% ($n = 13$) to tetracycline and 12% ($n = 8$) to ceftriaxone. Among the non-invasive PNSP, 49% ($n = 148$) were co-resistant to SXT, 44% ($n = 132$) to erythromycin, 35% ($n = 104$) to tetracycline and 21% ($n = 63$) to ceftriaxone (Fig. 2).

Seventy-two percent ($n = 86$) of the invasive ERP and 3% ($n = 4$) of the non-invasive ERP isolates were only resistant to erythromycin and were susceptible to all other tested antimicrobial agents (Fig. 2). In contrast, 13% ($n = 16$) of the invasive isolates and 85% ($n = 118$) of the non-invasive isolates were resistant to three or more antimicrobial agents (Fig. 2). Among the invasive ERP isolates, 18% ($n = 21$) were co-resistant to SXT, 13% ($n = 16$) to penicillin and 11% ($n = 13$) to tetracycline. Among the non-invasive ERP isolates, 95% ($n = 132$) were co-resistant to penicillin, 69% ($n = 96$) to tetracycline, 49% ($n = 68$) to SXT and 29% ($n = 41$) to ceftriaxone (Fig. 2).

There was a remarkable difference between invasive and non-invasive isolates in the number of resistance markers. For both PNSP as well as ERP isolates, invasive isolates were more likely to be resistant to one or two antimicrobial agents whereas non-invasive isolates were likely to be resistant to three or more antimicrobial agents (Fig. 2). However, if an isolate carried co-resistance, both invasive and non-invasive isolates were likely to be co-resistant to the same antimicrobial agents: penicillin/erythromycin, SXT, tetracycline and ceftriaxone (Fig. 2).

Serotypes with a high number of co-resistance markers were serotypes 14, 19F, 6B and NT, the same serotypes as the dominant non-susceptible serotypes, and these types were also characterised by having a high number of resistance profiles (data not shown). The fact that these isolates were very diverse indicates that non-susceptible isolates arose from several single genetic changes. Accumulation of such single genetic changes may be more likely to occur and persist in selective environments where the changes provide an advantage.

Non-invasive non-susceptible isolates were more likely to be non-susceptible to three or more antimicrobial agents than invasive non-susceptible isolates. In addition, non-invasive PNSP isolates were observed to have a higher serotype diversity than invasive PNSP isolates. A limited number of serotypes were shown to be dominant both among invasive and non-invasive non-susceptible isolates, which were also the serotypes with a high number of co-resistances to other antimicrobial agents and different resistance profiles. Why these changes causing non-susceptibility to

antimicrobial agents occur in only a few serotypes may be due to a combination of reasons. First, these serotypes are among the most prevalent types. Second, these serotypes may be more likely to gain, accumulate or take advantage of genetic changes and, third, these types may be present more often in environments with antibiotic pressure than other types, as these serotypes are also the ones commonly found as carriage isolates. Carriage isolates may be subject to antibiotic pressure more often than isolates with a highly invasive potential as they may be exposed to subtherapeutic concentrations of antibiotics during treatment of an infection.

With this knowledge that non-invasive pneumococcal isolates are more often resistant to several antimicrobial agents than invasive isolates because they may more often be exposed to antibiotic pressure as well as that the serotype distribution of the invasive and non-invasive non-susceptible isolates is overlapping, the optimal surveillance programme for antibiotic resistance among pneumococci should include surveillance of antimicrobial agents used for treatment of pneumococcal infections. Antibiotic susceptibility should be monitored both among invasive and a well defined subset of non-invasive and carriage isolates. In addition, information from general practitioners on the indications of antibiotic for treating infections would be useful to be able to follow the consumption of antimicrobial agents for respiratory tract infections and to evaluate whether the use of specific antimicrobial agents is more likely to cause resistance in specific pneumococcal serotypes than others.

Acknowledgments

The authors thank the Danish departments of clinical microbiology for sending isolates to the National *Neisseria* and *Streptococcus* Reference Center (Copenhagen, Denmark). Staff in the laboratories are thanked for their technical expertise and positive commitment to the work. Part of this study was presented at the 6th International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD-6), 8–12 June 2008, Reykjavik, Iceland.

Funding

This work was part of the national surveillance of severe streptococcal infections as well as part of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP), both funded by the Danish Ministry of the Interior and Health.

Competing interests

None declared.

Ethical approval

Not required.

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Fig. 1. Number of pneumococcal isolates of each serotype in relation to the number of antimicrobial agents to which non-susceptibility was detected. PNSP, penicillin-non-susceptible pneumococci; ERP, erythromycin-resistant pneumococci.

Fig. 2. Number of pneumococcal isolates with each resistance profile in relation to the number of antimicrobial agents to which non-susceptibility was detected. PNSP, penicillin-non-susceptible pneumococci; ERP, erythromycin-resistant pneumococci; PEN, penicillin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; ERY, erythromycin; CRO, ceftriaxone; CIP, ciprofloxacin; CHL, chloramphenicol.

