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ORIGINAL ARTICLES

Surveillance report

SURVEILLANCE OF ANTIMICROBIAL RESISTANCE OF INVASIVE PATHOGENS: THE ESTONIAN EXPERIENCES

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The aim of the present study was to evaluate the needs for surveillance of invasive Gram-negative pathogens in Estonia. The antimicrobial susceptibility data of invasive isolates of *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella spp, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae* and *enterococci* were collected in accordance with EARSS (European Antimicrobial Resistance Surveillance System) protocols. Despite the higher rate of Gram positive pathogens, their resistance was low in contrast to the elevated resistance established for Gram negative pathogens. The higher resistance to antimicrobials was particularly associated with *A. baumannii* and *P. aeruginosa*. Also, the proportion of extended-spectrum beta-lactamases (ESBL)-producing strains was 23% among *Klebsiella spp.* and 3.6% among *E. coli*. The inclusion of invasive Gram negative pathogens in antimicrobial resistance surveillance provides useful information concerning local pathogen susceptibility, as well as for the empirical treatment of suspected infections.

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Introduction

The epidemiology of invasive bloodstream pathogens has changed dramatically over the years [1-3]. The change in the incidence and epidemiology of infecting organisms has also brought about an increase in resistance to many antibiotic compounds [2,4,5]. Despite numerous publications on antimicrobial resistance, the comparison and evaluation of data is difficult, as the patient groups, sampling sites and infections involved in each study were different.

In order to overcome these problems, the European Antimicrobial Resistance Surveillance System (EARSS) began the collection of standardised data about the resistance of invasive isolates, focusing especially on Gram positive pathogens. Until 2005, information about Gram negative bacteria was available only in case of *E. coli* [6]. In addition, from the summer of 2005 onwards, data are being collected on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [6]. Infections with Gram negative bacteria still constitute a topical problem in patients with invasive infections, which are quite frequent in Europe [7-13].

The high degree of cultural, economic and social diversity, as well as the habits of antibiotic usage in European countries, probably influence the spectrum and susceptibility pattern of invasive pathogens, for example, the variation in the number of antibiotic prescriptions per 1000 population as well as the choice/preference of different antibiotic groups between the northern, central and eastern European countries was found [14,15,16,17]. Treatment and infection control guidelines also vary between countries [17]. Hence the usefulness of the resistance markers traditionally used in surveillance (such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and penicillin-nonsusceptible *Streptococcus pneumonia*) may have limited value for empirical antibiotic therapy and the evaluation of resistance trends in some regions. The aim of this study was to use the EARSS protocols and network to introduce surveillance of the resistance of invasive Gram negative pathogens and to evaluate their resistance and importance, in addition to studying the pathogens traditionally dealt with by EARSS.

Methods

The antimicrobial susceptibility data of invasive (blood and cerebrospinal fluid) non-duplicated isolates of *Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella spp., Escherichia coli, S. aureus, S. pneumoniae* and *enterococci* were collected between March and December 2004 at ten Estonian hospitals participating in EARSS. Since these hospitals include all hospitals performing blood cultures, the catchment population is almost all of Estonia's 1.4 million population. Two culture systems were used: Bactec (Becton Dickinson, USA, six hospitals) and Signal System (Oxoid, UK, four hospitals). For background data about the hospitals, number of samples and percentage of positive cultures and their nomenclature (non-duplicated analyses only) was collected from January to December 2004.

Gram negative pathogens were tested for meropenem, ceftazidime, cefepime, ampicillin/sulbactam, piperacillin/tazobactam, amikacin and ciprofloxacin by E-test (AB Biodisk, Solna, Sweden), according to the manufacturer's instructions. In order to determine extended spectrum betalactamase (ESBL) producers, an E test with ceftazidime and ceftazidime combined with clavulanic acid was used. The susceptibility of Gram positive bacteria was established on the basis of EARSS protocols [6].

The study protocol was approved by the ethics committee of the Estonian Institute of Experimental and Clinical Medicine (2004).

Table 1

Spectrum of invasive pathogens isolated in Estonia, January-December 2004

Pathogens (n=1315)	Total	Percentage
Gram negative pathogens	433	33%
E. coli	174	13.2
Enterobacteriaceae	87	6.6
Klebsiella spp.	61	4.6
Gram negative nonfermenters	48	3.7
P. aeruginosa	36	2.7
A. baumannii	27	2
Gram positive pathogens	824	62.7%
CONS	470	35.7
S. aureus	113	8.6
Streptococci	88	6.7
Enterococci	76	6
S. pneumoniae	49	3.7
Gram positive aerobic rods	28	2
Anaerobes	19	1.5
Pathogenic fungi	39	3

Results

Ten hospitals with between 160 and 942 beds (mean 487) and a total of between 48 291 and 272 169 patient days (total 1 297 246) per year participated in the prospective study. The number of collected samples (blood bottles) per 100 patient days varied from 0.1 to 3.2 (median 1.6 per 100 patient days). In total, 19 648 invasive samples were examined and 1315 non-duplicate invasive isolates were isolated from blood and cerebrospinal fluid in 2004 [TABLE 1]. The median proportion of positive samples was 12% (ranges 4.6-16.4%).

The majority were coagulase-negative staphylococci (CONS, 35.7%) followed by E. coli (13.2%) and *S. aureus* (8.6%). Among the Gram negatives, other Enterobacteriaceae accounted for 6.6%, *Klebsiella spp.* 4.6%, other Gram negative non-fermenters 3.7%, *P. aeruginosa* 2.7% and *A. baumannii* 2%. Among the Gram positives, the share of *S. pneumoniae* was 3.7%, the share of enterococci 6% and the share of other streptococci 6.7%.

A subset of 216 Gram negative pathogens were collected during the study period, including 117 *E.coli*, 56 *Klebsiella spp.*, 29 *P. aeruginosa*, and 14 *A. baumannii* strains [TABLE 2]. The isolates of E.coli and Klebsiella spp were susceptible to meropenem and amikacin, resistance to ciprofloxacin was 3% and 11% respectively. The higher resistance to antimicrobials was associated particularly with *A. baumannii* and *P. aeruginosa*. Also, the proportion of ESBL-producing strains was 23% among *Klebsiella spp.* and 3.6% among *E. coli*.

Table 2

$MIC_{50/90}$ values and percentage of susceptibility of *A. baumannii, P. aeruginosa, Klebsiella* spp, and *E.coli* invasive strains isolated in Estonian hospitals, March-December 2004

Antibiotic	Pathogen $MIC_{50/90}$ (% of susceptibility)			
	A. baumannii N=14	*P. aeruginosa* N=29	*Klebsiella* spp. N=56	*E. coli* N=117
Ampicillin sulbactam	4/16 (64)	ND	ND	ND
Piperacillin tazobactam	ND	6/64 (89)	ND	ND
Cefepime	6/16 (71.4)	ND	ND	ND
Ceftazidime	ND	1.5/8 (91.4)	ND	ND
Meropenem	1.5/4 (100)	1/6 (86)	0.032/0.064 (100)	0.023/0.032 (100)
Amikacin	3/256 (78.6)	3/12 (96.6)	2/3 (98)	2/3 (100)
Ciprofloxacin	ND	0.19/6 (83)	0.032/1 (89)	0.016/0.047 (96.7)

Overall antimicrobial resistance among major bloodstream pathogens in Estonia was relatively low in the case of Gram positive indicator pathogens. No penicillin non-susceptible S. pneumoniae were found. The proportion of methicillin-resistant *S. aureus* was 4%, and the proportion of vancomycin non-susceptible enterococci was 1.6% (one strain with MIC value 6 mL).

Discussion

The most frequent invasive pathogens were coagulase-negative staphylococci, *E. coli* and *S. aureus*. Similarly, the five most common pathogens in other European studies were also *E. coli*, *S. aureus*, CONS, enterococci and *Klebsiella spp.* [8,10,11]. In our study, the ratio of Gram positive to Gram negative pathogens was 1.9. According to the data from the literature, Gram negative bacilli were the predominant pathogens in the 1970s; in recent decades, Gram positive cocci, especially CONS, have emerged as a more frequent cause of invasive infections [1-3,18]. The increase in CONS could be attributed to the increasing proportion of neonatal and haematological patients. However, the quantity of

true infections and contamination is impossible to evaluate, since harmonised exclusion algorithms for common skin contaminants are not used in our study or other published studies.

Antimicrobial resistance among Estonian invasive pathogens was relatively low, more closely resembling northern European than southern and eastern European regions [19]. This is especially true in the case of Gram positive pathogens [6-8,10,20]. However, the isolation of the first strains of VRE and the recent increase of MRSA cases in some Estonian hospitals may predict an emergence of resistance [6].

Despite the relatively lower frequency of *A. baumannii* and *P.aeruginosa*, the higher resistance to antimicrobials was particularly associated with these pathogens, and this is similar to the experience of other authors [7-10,12,21]. A comparison of the data from the SENTRY and MYSTIC study with those from Estonia shows some differences in antibiotic choice and study criteria and the limitations of pooling those data. In general, Gram negative invasive isolates from Estonian hospitals were at least as sensitive as the European average [6,8].

The use of invasive strains in resistance surveillance has some advantages. The inclusion criteria are clear, and since colonisation and contamination are excluded (except CONS), these strains are real pathogens, making the data more comparable. Since the number of strains is relatively small, more expensive but also more informative methods, such as MIC detection and typing, can be used. However, different sampling habits between different hospitals and countries may influence the quality of the data [6]. It is also not clear how the resistance of invasive strains represents the overall situation of proportions and trends. Today, few studies with controversial results [13,19,22] are available offering comparative information about the aetiology and susceptibility of both invasive as well as non-invasive pathogens.

It is a common view that resistance surveillance should focus mainly on MRSA and other Gram positive organisms. In our situation, however, where high resistance and therapy failures are frequently associated with Gram negative bacteria (such as Klebsiella, Acinetobacter and Pseudomonas), the inclusion of these pathogens for antimicrobial resistance surveillance provides useful information [6,23].

Thus we can conclude that due to interstate and regional (for example, eastern, central and northern Europe) differences in pathogens' profile and susceptibility pattern, international conventional surveillance systems should be modified according to local situations, and additional diagnostic methods should be included if necessary.

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