

Short communication

The comparison of susceptibility patterns of Gram-negative invasive and non-invasive pathogens in Estonian hospitals

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Accepted in revised form 21 November 2005

Key words: Gram-negative invasive and non-invasive pathogens, Susceptibility

Abstract

A total of 560 invasive and 1062 non-invasive isolates were collected. The antimicrobial susceptibility of invasive versus non-invasive *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* isolates were evaluated using the *E*-tests. The equal domination of Gram-negative among both invasive and non-invasive pathogens was estimated in our study if contaminants were excluded. The emergence trend of Gram-positive microbes especially of coagulase negative staphylococci may be proved only after application of exclusive algorithms. Due to similar susceptibility, the data of non-invasive Gram-negative pathogens can be useful to predict resistance of invasive ones. Also, the surveillance of invasive pathogens provides useful information about the general susceptibility of pathogens.

Findings

Invasive pathogens are microorganisms isolated from blood and cerebrospinal fluid (European Antimicrobial Resistance Surveillance System. <http://www.earss.rivm.nl>). The invasive infections are common in critically ill patients especially in ICUs (intensive care units) where the different aetiology and antimicrobial susceptibility of the pathogens make the choice of essential empirical antibiotic therapy troublesome. According to the literature the most common sources of invasive infection are the respiratory tract and an intra-

vascular line (Crowe et al. 1998; Urli et al. 2002). Via translocation those pathogens may frequently invade the bloodstream, being closely related to such severe conditions as systemic inflammatory response syndrome, sepsis, and shock.

Severe problems with a high rate of resistance as well as the risk of spread of these resistant bacteria are frequent in ICUs (Itokazu et al. 1996; Leroyer et al. 1997), having relevant influence on morbidity and mortality (Vincent 2003). Especially problems with a high rate of resistance as well as the risk of spread of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* are frequent

in ICUs (Itokazu et al. 1996; Leroyer et al. 1997; Weber et al. 1999; Canton et al. 2003). Today few studies with controversial results (Jones et al. 2004; Lau et al. 2004; Unal et al. 2004) are available giving comparative information about the aetiology and susceptibility of both invasive as well as non-invasive pathogens.

To overcome these problems EARSS (European Antimicrobial Resistance Surveillance System) started the collection of standardized data about resistance of invasive isolates (i.e. true infection strains), but it is not known whether these data represent the whole resistance situation on community, hospital, ICU as well as country level. On the other hand there is no estimate of how much ICU routine susceptibility data (e.g. tracheal colonisers) can predict resistance of the invasive bacteria in septic patients.

For this purpose the aim of our study was to assess the comparative data about the total aetiology of invasive and non-invasive strains, the susceptibility pattern of both non-invasive and invasive strains most commonly interrelated with elevated resistance (*A. baumannii*, *P. aeruginosa*, *K. pneumoniae*), and thereby to give putative treatment guidelines based on Estonian experience.

Two regional Estonian hospitals were included (Tartu University Clinics and North Estonian Regional Hospital) in prospective periodic surveillance performed in 2003 (collection of non-invasive strains isolated from tracheal aspirate and sputum of *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* from ICUs only), and in 2004 (collection of all the same invasive strains isolated from blood and cerebrospinal fluid) from both hospitals). An isolate was defined as the same species of bacteria with the same antimicrobial susceptibility pattern isolated from the same patient. Organisms identified by the participating centres using the identification method routinely employed in their clinical microbiology laboratory (Murray et al. 2003, European Antimicrobial Resistance Surveillance System). For invasive pathogens the commercial API System (Bio-Mérieux, France) was additionally employed. Minimum inhibitory concentration (MIC) was determined in each centre using *E*-tests according to the manufacturer's instructions (AB Biodisk, Solna, Sweden). Interpretative criteria were those recommended by the National Committee for

Clinical Laboratory Standards (NCCLS 2003). To determine ESBL (Extended Spectrum Betalactamase) producers an *E*-test with ceftazidime and ceftazidime combined with clavulanic acid was used.

For background, data on total structure of invasive and non-invasive pathogens (non-duplicated analyzes only) was collected.

Quality control was performed with the following strains recommended by NCCLS: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *K. pneumoniae* ATCC 700603.

The prevalence of bacterial groups and proportions of susceptible and resistant strains were compared using χ^2 test, and for comparison of MIC values Mann–Whitney Rank Sum test or *t*-test was used (Jandel SigmaStat 2.0 programme).

The Ethics Review Committee on Estonian Institute of Experimental and Clinical Medicine approved our study protocol (2003 and 2004).

In the prospective study North Estonian Regional Hospital (NERH) and Tartu University Clinics (TUC) were participating with total bed-sites 1764 (63 in ICUs) and a total of 493 397 patient days in hospital and 11 624 in ICUs, respectively. The percentage of positive invasive samples was 12% and the samples per 100 patient days were 2.96. The percentage of positive non-invasive samples was 39% and the samples per 100 patient days were 52 in ICUs.

A total of 560 invasive and 1062 non-invasive isolates were collected. Of the non-invasive isolates 28% were Gram-positive and 58% Gram-negative. In contrast, 64% of the invasive isolates were Gram-positive and 32% Gram-negative bacteria, whereas coagulase negative staphylococci (CONS) comprised 39% of all pathogens. Generally, if CONS (Weinstein 2003) as frequent contaminants were excluded, both invasive and non-invasive pathogens were mostly Gram-negative bacteria (Figure 1). The aetiology of non-invasive versus invasive pathogens was as follows: Gram-positive microbes 23/41%, Gram-negative rods 62/52%, anaerobes 3/2%, and pathogenic fungi 12/5%. Pathogenic fungi were also more frequently isolated from non-invasive materials ($P < 0.05$). The prevalent non-invasive species in Estonian ICUs were *K. pneumoniae*, pathogenic fungi and *Enterobacteriaceae*, *S. aureus*, *P. aeruginosa* and *A. baumannii*. Among invasive pathogens *E. coli*, streptococci (beta-haemolytic streptococci 30%,

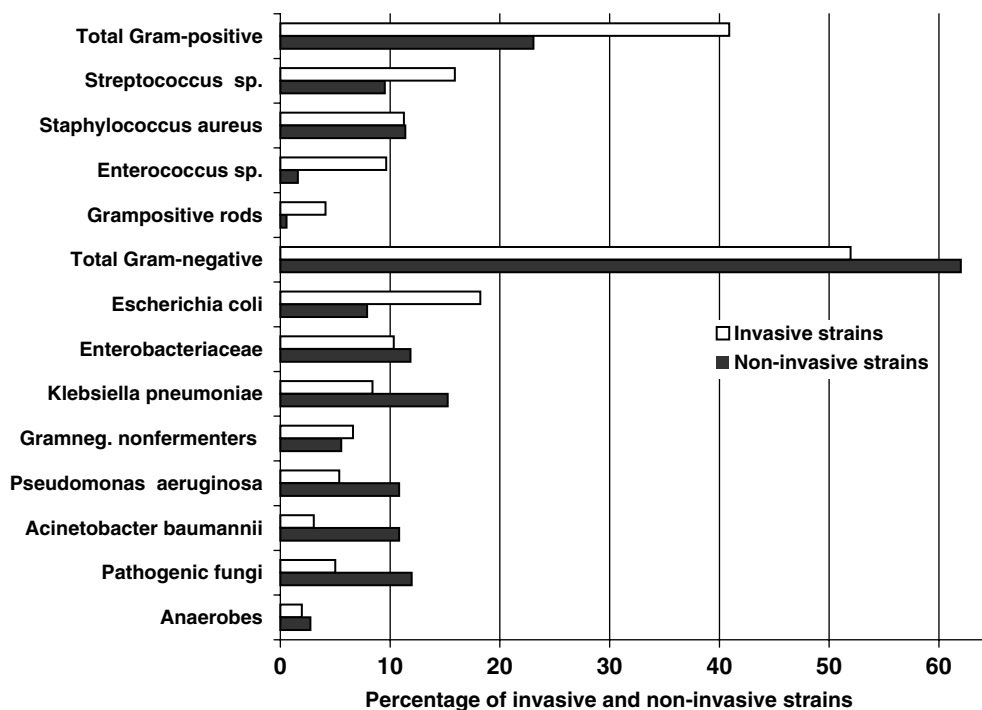


Figure 1. The structure of invasive and non-invasive pathogens.

S. pneumoniae 28%, and Viridans-streptococci 42%) and *S. aureus* were in the leading position similar to the European data (Fluit et al. 2000; Assadian et al. 2002; Luzzaro et al. 2002). In our study the majority of invasive strains originated from ICU patients, explaining the equal ratio of Gram-negative both invasive and non-invasive pathogens.

However, according to the literature Gram-negative bacilli were the predominant pathogens in the 1970s; Gram-positive cocci, especially CONS have emerged as a more frequent cause of invasive infections during recent decades (Klastersky 1998; Karchmer 2000; Karlowsky et al. 2004). Increase of CONS could be attributed to the increasing rate of neonatal and haematological patients, but the amount of real infections and contamination is impossible to evaluate, since common algorithms for exclusion of probable contaminants are unavailable. The aforementioned majority of invasive strains as well as CONS originated from adult ICUs. Unfortunately it was not feasible to determine whether the isolates caused infection or reflected colonization in those patients. Thus critical analysis of invasive CONS isolates and appli-

cation of strict algorithms is a prerequisite for estimation of a real increase of Gram-positive pathogens.

A total of 340 Gram-negative pathogens, including 95 *A. baumannii*, 108 *P. aeruginosa*, and 137 *K. pneumoniae* strains were tested, as depicted in Table 1. The isolates of *K. pneumoniae*, derived from blood samples showed higher MIC values to meropenem and amikacin ($P < 0.05$). However, the proportions of resistant and susceptible strains were not statistically different ($P > 0.05$). ESBL-positive *K. pneumoniae* strains were found to be non-invasive 18% and invasive 19% respectively. According to the literature few and controversial data are available whereas some authors state that invasive pathogens are more (Jones et al. 2004) or less resistant (Lau et al. 2004) than non-invasive ones. In concordance with pan-European findings (Unal et al. 2004) we estimated no differences in susceptibility pattern of pathogens derived from both sources.

The antimicrobial resistance among Estonian invasive pathogens was relatively lower resembling North Europe than South and East regions (Unal et al. 2004). The resistance to antimicrobials was closed with Gram-negatives, especially

- isolates from nine surgical intensive care units, 1996–2000. *Eur. J. Clin. Microbiol. Infect. Dis.* 21: 743–747.
- Canton R., Coque T.M. and Baquero F. 2003. Multi-resistant Gram-negative bacilli: from epidemics to endemics. *Curr. Opin. Infect. Dis.* 16: 315–325.
- Crowe M., Ispahani P., Humphreys H., Kelley T. and Winter R. 1998. Bacteraemia in the adult intensive care unit of a teaching hospital in Nottingham, UK, 1985–1996. *Eur. J. Clin. Microbiol. Infect. Dis.* 17: 377–384.
- Daxboeck F., Assadian O., Blacky A., Koller W. and Hirschl A.M. 2004. Resistance of Gram-negative non-fermentative bacilli causing bloodstream infection, Vienna, 1996–2003. *Eur. J. Clin. Microbiol. Infect. Dis.* 23: 415–416.
- European Antimicrobial Resistance Surveillance System. <http://www.earss.rivm.nl>. Accessed 22 November 2005.
- Fluit A., Jones M.E., Schmitz F.J., Acar J., Gupta R. and Verhoef J. The SENTRY Participants Group 2000. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY Antimicrobial Surveillance Program, 1997 and 1998. *Clin. Infect. Dis.* 30: 454–460.
- Itokazu G.S., Quinn J.P., Bell-Dixon C., Kahan F.M. and Weinstein R.A. 1996. Antimicrobial resistance rates among aerobic Gram-negative bacilli recovered from patients in intensive care units: evaluation of a national postmarketing surveillance program. *Clin. Infect. Dis.* 23: 779–784.
- Jones M.E., Karlowsky J.A., Draghi D.C., Thornsberry C., Sahm D.F. and Bradley J.S. 2004. Rates of antimicrobial resistance among common bacterial pathogens causing respiratory, blood, urine, and skin and soft tissue infections in pediatric patients. *Eur. J. Clin. Microbiol. Infect. Dis.* 23: 445–455.
- Karchmer A.W. 2000. Nosocomial bloodstream infections: organisms, risk factors, and implications. *Clin. Infect. Dis.* 31: 139–143.
- Karlowsky J.A., Jones M.J., Draghi D.C., Thornsberry C., Sahm D.F. and Volturo G.A. 2004. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. *Ann. Clin. Microbiol. Antimicrob.* 3: 7 Accessed at <http://www.ann-clinmicrob.com/content/3/1/7>.
- Klastersky J. 1998. Science and pragmatism in the treatment and prevention of neutropenic infection. *JAC* 41: 13–24.
- Lau S.M., Peng M.Y. and Chang F.Y. 2004. Resistance rates to commonly used antimicrobials among pathogens of both bacteremic and non-bacteremic community-acquired urinary tract infection. *J. Microbiol. Immunol. Infect.* 37: 185–191.
- Leroyer A., Bedu A., Lombrail P., Desplanques L., Diakite B. and Bingen E. 1997. Prolongation of hospital stay and extra cost due to hospital-acquired infection in a neonatal unit. *J. Hosp. Infect.* 35: 37–45.
- Luzzaro F., Viganò E.F., Fossati D., Grossi A., Sala A. and Sturla C. AMCLI Lombardia Hospital Infectious Study Group 2002. Prevalence and drug susceptibility of pathogens causing bloodstream infections in northern Italy: a two-year study in 16 hospitals. *Eur. J. Clin. Microbiol. Infect. Dis.* 21: 849–855.
- Murray RE, Baron EJ, Jorgensen JH, Pfaller MA and Tenover FC 2003. *Manual of Clinical Microbiology* 8th ed. ASM Press, Washington, DC.
- National Committee for Clinical Laboratory Standards. 2003. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Sixth Edition.* 20 (2).
- Unal S., Masterton R. and Goossens H. 2004. Bacteraemia in Europe – antimicrobial susceptibility data from the MYSTIC surveillance programme. *Antimicrob. Agents* 23: 55–63.
- Urli T., Perone G., Acquarolo A., Zappa S., Antonini B. and Ciani A. 2002. Surveillance of infections acquired in intensive care: usefulness in clinical practice. *J. Hosp. Infect.* 52: 130–135.
- Vincent J. 2003. Nosocomial infections in adult intensive-care units. *Lancet* 361: 2068–2077.
- Weber D.J., Raasch R. and Rutala W.A. 1999. Nosocomial infections in ICU. The growing importance of antibiotic-resistance pathogens. *Chest* 115: 34–41.
- Weinstein M.P. 2003. Blood culture contamination: persisting problems and partial progress. *J. Clin. Microbiol.* 41(6): 2275–2278.