Original Article

Trends in antimicrobial susceptibility of Gram-negative bacteria isolated from blood in Jakarta from 2002 to 2008

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Abstract

Background: This study examined the susceptibility of Gram-negative bacteria in the bloodstream to antimicrobials with the aim of providing information relevant to the guidance of therapy.

Methodology: Blood specimens received by the Laboratory of Clinical Microbiology, Faculty of Medicine, University of Indonesia, from 2002 to 2008, were analyzed for the presence of Gram-negative bacteria and their susceptibility to four antibiotic groups frequently administered in hospitals and community settings.

Results: During the seven-year period leading up to 2008, approximately 68% of Gram-negative bacteria were identified among all positive isolates from blood specimens. The eight most frequent species found were *Acinetobacter anitratus* (25.8%), *Pseudomonas aeruginosa* (19.5%), *Klebsiella pneumoniae* subsp. pneumoniae (14.5%), *Enterobacter aerogenes* (8%), *Salmonella* Typhi (7.5%), *Escherichia coli* (6.2%), *Alcaligenes faecalis* (5.6%) and *Klebsiella oxytoca* (3.2%). At 80% susceptibility or greater, Ceftriaxone and Cefotaxime were active only on *E. coli* and *S.* Typhi. Cefepime demonstrated activity on all eight species tested except *K. pneumonia* while Amikacin showed activity against five species, *A. faecalis*, *E. aerogenes*, *E. coli*, *K. pneumoniae* subsp. pneumoniae and *S.* Typhi. Gentamycin was active against three species: *E. aerogenes*, *K. oxytoca* and *S.* Typhi. Ciprofloxacin and Levofloxacin significantly differed in their spectrum: while Ciprofloxacin was active against four of the eight species tested (*E. aerogenes*, *E. coli*, *K. oxytoca*, and *S.* Typhi), Levofloxacin was similar to Cefepime and was active against all eight species except *K. pneumoniae* subsp. pneumonia.

Conclusions: Since antimicrobials are broadly used in Jakarta, it is important that the information captured in this study be disseminated.

Key words: Gram-negative bacteria, susceptibility to antibiotics

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Introduction

Infections caused by Gram-negative bacteria remain a worldwide problem. Two thirds of septic shock cases are caused by Gram-negative bacteria such as *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Pseudomonas*, and *Bacteroides* with the remainder being caused by Gram-positive bacteria such as *Streptococcus pneumonia*, *Streptococcus pyogenes*, and *Staphylococcus aureus* [1]. In the United States, Gram-negative organisms were the leading cause of health-care associated blood stream infections before the 1970s, while Gram-positive organisms have since been the predominant microbial isolates [2]. An estimated 200,000 Americans develop Gram-negative sepsis each year with reported mortality rates of 30% to 65% [3]. Gram-negative sepsis is associated with a mortality rate three times greater than Gram-positive sepsis [4]. In India, Pseudomonas sp. and *E. coli* accounted for approximately 20% of 567 positive isolates derived from patients with fever and sepsis while Citrobacter, Acinetobacter, Proteus, Enterobacter spp. collectively accounted for 80.96% [5].

The present study reported Gram-negative organisms isolated from blood specimens analyzed at the Clinical Microbiology Laboratory of the Faculty of Medicine, University of Indonesia (CML-FMUI), from 2002 to 2008 and their antimicrobial susceptibility patterns. The variation of antimicrobial susceptibility patterns is an important factor in health care awareness associated with managing infectious disease patients and in minimizing the emergence and spread of multidrug-resistant microorganisms.

Materials and methods

Specimens

The present study examined all blood specimens received by the Laboratory of CML-FMUI in Jakarta from hospitals, private practices, or individuals from 1January 2002 to 31 December 2008.

Cultures and antibiotic susceptibility tests

Cultures and susceptibility tests to antibiotics were performed according to CML-FMUI standard practices [6,7] and Performance Standards for Antimicrobial Susceptibility Testing from the Clinical and Laboratory Standards Institute (CLSI) [8]. Cultures were performed using Bactec 9050 (Becton Dickinson) and microorganism identification was determined using standard biochemical reactions. The susceptibility of microorganisms to antibiotics was assessed using the disc diffusion method. Antimicrobial susceptibility results were categorized in to three groups: Sensitive (S), Intermediate (I) and Resistant (R) according to CLSI [8]. The antibiotics susceptibility data was then entered into the WHO-Net program.

Antibiotics tested

The following antibiotic discs were included in the present study: Ceftriaxone (30 µg), Cefotaxim (30 µg), Cefepime (30 µg), Levofloxacin (5 µg), Ciprofloxacin (5 µg), Amikacin (30 µg) and Gentamycin (10 µg). Assessments were made based on Gram-negative isolates and numbers of isolates ≥ 10. Good activity *in vitro* was defined by antimicrobial susceptibility of 80% or greater for Gram-negative bacteria.

Results

A total of 687 isolates were found in blood specimens analyzed in the targeted 2002 to 2008 time period , with 461 of these isolates being Gram-negative bacteria (67.1%). Eight species of Gram-negative bacteria with a total number of 418 isolates (90.7%) met the present study's criteria. Their antibiotic susceptibility is listed in Table 1. *Acinetobacter anitratus*, accounting for 25.8% of the isolates, was the predominant species identified during the assessed time period, followed by *Pseudomonas aeruginosa* at 19.5 %, *Klebsiella pneumoniae* subsp. pneumoniae at 14.5 %, *Enterobacter aerogenes* at 8 %, *Salmonella* Typhi at 7.5 %, *E. coli* at 6.2 %, *Alcaligenes faecalis* at 5.6 %, and *Klebsiella oxytoca* at 3.2 %.

The spectrum of tested anti-mirobials per type of bacteria is listed in Table 2. Ceftriaxone and Cefotxim was found to be active on only two species of bacteria, *E. coli* and *S.* Typhi. Remarkably, Cefepime and Levofloxacin were found to be active on all eight bacterial species except for *K. pneumoniae*. Amikacin showed good activity on five species of bacteria: *A. faecalis, E. aerogenes, E. coli*, *K. pneumoniae* and *S.* Typhi. Ciprofloxacin showed activity against four bacterial species: *E.aerogenes*, *E. coli*, *K. oxytoca*, and *S.* Typhi. In comparison, Gentamycin was found to be active on only three species of bacteria: *E. aerogenes*, *K. oxytoca* and *S.* Typhi.

A. anitratus and P. aeruginosa were less susceptible to Ceftriaxone, Cefotaxime, Amikacin, Gentamycin and Ciprofloxacin than to Cefepime and Levofloxacin. *A. faecalis* showed good sensitivity to three antibiotics, i.e. Cefepime, Amikacin and Levofloxacin, while *E. aerogenes* exhibited good sensitivity to almost all the antibiotics analyzed, i.e. Cefepime, Amikacin, Gentamycin, Ciprofloxacin, and Levofloxacin. *E. coli* was sensitive to all antibiotics tested except Gentamycin. *K. pneumoniae* showed very poor susceptibility to all antibiotics except Amikacin. *S.* Typhi showed good sensitivity to all antibiotics tested.

Discussion

The management of patients with infectious diseases must focus on patient safety, which includes minimizing the emergence and spread of resistant strains and deciding on the appropriate antimicrobial to administer. Moreover, in sepsis cases, the focal site(s) of infection should be identified to determine which bacteria are involved [9]. In cases with unknown focal site(s) of infection, empiric therapy should be based on the likeliest sources of bacteremia [10].

Ceftriaxone, a third generation Cephalosporin, has broad-spectrum activity against Gram-positive and Gram-negative bacteria. In general, it is considered to be equivalent to Cefotaxime, in terms of safety and efficacy. Ceftriaxone and Cefotaxime are indicated for sepsis syndrome, as well as for pneumonia and skin infection [9]. In our study, Ceftriaxone and Cefotaxim showed less activity with almost all Gram-negative bacteria isolates except for *E. coli* and *S.* Typhi. These results are similar to those from a study conducted in India in which it was found that approximately 50% of fermenter and non-

Table 1. Percentage of eight species Gram-negative bacteria susceptible to antimicrobials in Jakarta from 2002 to2008.

No.	Organisms	No. Isolates	Susceptible to (%)						
			CRO	CTX	FEP	AMK	GEN	CIP	LVX
1.	*Acinetobacter anitratus*	*119 (25.8%)*	*50.4*	*79*	*89.9*	*57.1*	*34.4*	*68.9*	*82.3*
2.	*Alcaligenes faecalis*	*26 (5.6%)*	*27.3*	*53.8*	*88.5*	*87.5*	*26.9*	*76*	*100*
3.	*Enterobacter aerogenes*	*37 (8%)*	*31.6*	*68*	*94.4*	*100*	*81.1*	*91.9*	*95.8*
4.	*Escherichia coli*	*29 (6.2%)*	*86.2*	*90*	*86.2*	*82.7*	*68.9*	*82.7*	*82.7*
5.	*Klebsiella oxytoca*	*15 (3.2%)*	*0*	*69.2*	*80.0*	*NE*	*86.7*	*86.7*	*93.3*
6.	*Klebsiella pneumonia subsp. pneumoniae*	*67 (14.5%)*	*32.8*	*46.7*	*76.1*	*98.5*	*50.7*	*59.7*	*76.1*
7.	*Pseudomonas aeruginosa*	*90 (19.5%)*	*42.2*	*55.1*	*88.9*	*56.9*	*27.8*	*77.8*	*92.2*
8.	*Salmonella* Typhi	*35 (7.5%)*	*92.6*	*86.7*	*94.1*	*100*	*100*	*100*	*100*
	TOTAL	***418 (90.7%)***							

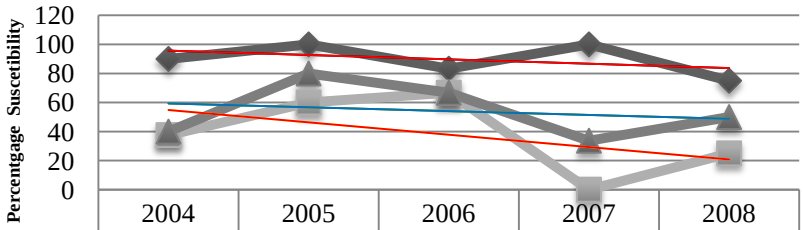
Parentheses refer to percentage of each species to total Gram negative bacteria isolated; CR0: Ceftriaxone, CTX: Cefotaxime, FEP: Cefepime, AMK: Amikacin, GEN: Gentamycin, CIP: Ciprofloxacin, LEV: Levofloxacin; NE: not evaluated

Table 2. Activity of the spectrum of antimicrobials tested on eight species of Gram-negative.

No.	Organisms	Antibiotics						
		CRO	CTX	FEP	AMK	GEN	CIP	LVX
1.	*Acinetobacter anitratus*			●				●
2.	*Alcaligenes faecalis*			●	●			●
3.	*Enterobacter aerogenes*			●	●	●	●	●
4.	*Escherichia coli*	●	●	●	●		●	●
5.	*Klebsiella oxytoca*			●		●	●	●
6.	*Klebsiella pneumoniae subsp. pneumoniae*				●			
7.	*Pseudomonas aeruginosa*			●				●
8.	*Salmonella* Typhi	●	●	●	●	●	●	●

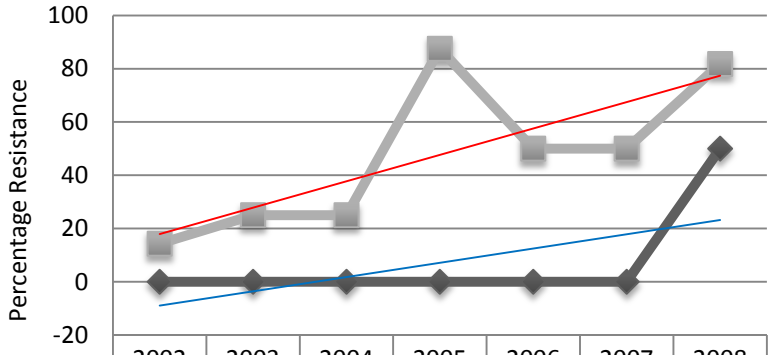
Black dots indicate good activity in vitro was defined as 80% or greater susceptibility of Gram-negative

Figure 1. Susceptibility of A. anitratus to Cefepime, Ceftriaxone and Gentamycin.



	2004	2005	2006	2007	2008
Cefepime	90	100	83,3	100	75
Ceftriaxone	37,5	60	66,7	0	25
Gentamycin	40	80	66,7	33,7	50

Figure 2. Resistance of *E. coli* and *K. pneumonia* to Ceftriaxone.



	2002	2003	2004	2005	2006	2007	2008
E. coli	0	0	0	0	0	0	50
K. pneumoniae	14,3	25	25	87,5	50	50	81,8

fermenter Enterobacteriaceae were susceptible to those antimicrobials [5]. In Europe, Canada and the United States in the years 2000 and 2001, *E. coli* and *K. pneumonia* exhibited approximately 90% susceptibility to both Ceftriaxone and Cefotaxim, while *Enterobacter spp.* demonstrated a lower susceptibility [9].

The present study showed that *P. aeruginosa* is intrinsically resistant to Ceftiaxone, Cefotaxim, Kanamycin and Neomycin. Similar to our results, *P. aeruginosa* showed less than 55% susceptibility to those antimicrobials in another study [9]. In European countries, Amikacin was active *in vitro* against *P. aeruginosa* (> 80% susceptibility) [9]; however, this was not so in India [5] and Indonesia (as found in the present study). Other commonly used Aminoglycoside antibiotics such as Gentamycin showed good activity against Gram-negative bacteria in Europe, but exhibited only 48% to Enterobacteriaceae in India. The present study found that more than 80% of *E. aerogenes*, *K. oxyyoca* and *S.* Typhi were susceptible to Gentamycin. Notably, Aminoglycoside has been found to be ineffective *in vivo* against *S.* Typhi despite its activity *in vitro*; therefore, it is not recommended for treatment of *S.* Typhi infections such as Typhoid Fever [8].

Cefepime is a fourth-generation Cephalosporin antibiotic which has an extended spectrum of activity against both Gram-positive and Gram-negative bacteria and has greater activity than third-generation agents. Cefepime is usually reserved for infections caused by *P. aeruginosa* and multidrug-resistant microorganisms. It is an effective antimicrobial of choice for critically-ill sepsis patients as well as patients with febrile neutropenia. Several studies have shown that Cefepime is associated with low

mortality rates, rapid clinical improvement, minimal drug resistance, and minimal adverse effects in patients with severe sepsis [12]. At present, Cefepime demonstrates good activity against Gram-negative bacteria *in vitro* including *P. aeruginosa*, which is consistent with the findings of the study. However there are issues surrounding the clinical safety of Cefepime as it has been associated with an increased mortality rate when used for different types of infections [13]. As a result, the administration of Cefepime is becoming less common.

Monotherapy has been associated with clinical failure in cases with serious infections caused by *P. aeruginosa*. For that reason, a combination treatment with beta lactam antimicrobials and Fluoroquinolone or Aminoglycoside should be considered [8]. In comparison to Ciprofloxacin, Levofloxacin exhibited superior activity in Gram-negative bloodstream isolates (see Table 2). Levofloxacin was active against almost all the Gram-negative bacteria assessed in this study, including *P. aeruginosa.* Ciprofloxacin did not exhibit similar activity. The results of the present study are consistent with those of Flynn *et al.* [14] which showed that the minimum inhibitory concentrations (MICs) for Levofloxacin against multi-resistant *P. aeruginosa* strains were generally two- to fourfold higher than those of Ciprofloxacin. *S.* Typhi exhibited good susceptibility to all the antibiotics tested; however, due to the low numbers of isolates tested in the present study, it is possible that antimicrobial-resistant *S.* Typhi strains were not found. It is not recommended that Aminoglycoside be used in treating *Salmonella* and *Shigella* infections as *in vitro* results did not correlate with *in vivo* conditions, and thus it was not clinically effective [8]. During the seven-year course of the present study, *A. anitratus* were the most commonly found isolates. *Acinetobacter* species are commonly recognized as commensal bacteria, but they have occasionally caused hospital infections. This microorganism was frequently found to be resistant to antimicrobial agents, and thus infections caused by *Acinetobacter* species were difficult to treat. In the present study *A. anitratus* demonstrated a decreasing susceptibility trend to third- and fourth-generation Cephalosporine antimicrobials (see Figure 1). *Klebsiella* species and *E. coli* were predominantly extended-spectrum beta lactamase (ESBL) producers, making these microorganisms clinically resistant to penicillins, Cephalosporines or Aztreonam despite apparent *in vitro* susceptibility. Results from the present study indicated an increasing number of *K. pneumonia* and *E. coli* strains resistant to Ceftriaxone from 2002 to 2008 (see Figure 2). In conclusion, Cefepime demonstrated superior *in vitro* activity for Gram-negative bacteria of the bloodstream compared to Ceftriaxone and Cefotaxim. Compared to Ciprofloxacin, Levofloxacin showed good activity against almost all the types of microorganisms found. Generally, Levofloxacin and Cefepime demonstrated good activity against both fermenter and non-fermenter Enterobacteriaceae. Since antibiotic susceptibility information is important in determining the guidelines for antibiotic therapy of infectious diseases, it is important that this information be collected on a regular basis.

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Conflict of Interest: No conflict of interest is declared.

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