ANTIBIOTIC RESISTANCE AND MORTALITY IN CHILDREN WITH NOSOCOMIAL BLOODSTREAM INFECTION IN A TEACHING HOSPITAL IN INDONESIA

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Abstract. Nosocomial infection is a major problem in hospitals worldwide. Understanding patterns of bacterial etiology and antibiotic susceptibility are important factors to combating nosocomial infection. Among children with nosocomial bloodstream infection (BSI), we identified pathogens and determined antibiotics resistance patterns and mortality rates for antibiotic-susceptible and multidrug-resistant (MDR) infection in patients with nosocomial BSI in pediatric wards and PICU at Dr Sardjito Hospital, Indonesia during December 2010 to February 2013. Of 174 isolates from 170 patients, 168 pathogens were bacteria, of which 148 were gram-negative. *Pseudomonas aeruginosa*, *Klebsiella* spp, Enterobacteriaceae, *Acinetobacter baumanii*, and *Escherichia coli* was found in 55%, 6%, 4%, 1%, and <1%, respectively of the isolates. Imipenem, amikacin, ciprofloxacin, and ceftazadime had the highest sensitivity to nosocomial pathogens at 86%, 84%, 84%, and 75%, respectively. Eleven patients had MDR-infections, 7 of whom died. Among 153 patients infected with bacteria resistant to <3 classes of antibiotics (non-MDR), mortality was 40%, and among 4 patients with fully drug-susceptible sepsis only one died. Thus, substantial mortality was observed in children with nosocomial-BSI, particularly with MDR pathogens. Given the further high risk of resistance with wider use of carbapenems, third generation cephalosporins and flouroquinolones, prevention should be given highest priority in combating hospital-acquired infection.

Keywords: antibiotic resistance, children, nosocomial bloodstream infection, developing countries, Indonesia

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INTRODUCTION

Nosocomial bloodstream infections carry high morbidity and mortality both in developed and developing countries. Nosocomial infection related to drug-

resistant bacteria is much more difficult to eradicate, and this infection is associated with considerable mortality (Ivady *et al*, 2015; Murni *et al*, 2015). Understanding the causes and antibiotic susceptibility of such pathogens is important in the fight against hospital-acquired infections. This information can help health workers make rational choices on the use of antibiotics and implement improved infection control practices.

The purposes of this study were to determine the antibiotic resistance patterns of nosocomial bloodstream infections and to determine mortality related to drug-sensitive, non-multidrug resistant (MDR), and MDR nosocomial infections. Although such data are most important locally, in many hospitals in Indonesia and other developing countries microbiological services are limited, and there is limited understanding of drug resistance patterns. This has masked the rise of antimicrobial resistance in developing countries.

MATERIALS AND METHODS

Study design and data collection

A prospective cohort study was conducted in pediatric wards and pediatric intensive care units **(**PICUs) at Dr Sardjito Hospital, Yogyakarta, Indonesia, between December 2010 and February 2013, analyzing antibiotic susceptibility data from patients with nosocomial bloodstream infection provided by the Clinical Pathology Laboratory. Blood cultures were taken when nosocomial bloodstream infection was suspected. Data collected included microbiological species from all patients with clinical features of nosocomial bloodstream infections. We analyzed bacterial isolates, antibiotic susceptibility results and antimicrobial resistance patterns.

Determination of antibiotics sensitivity

Blood cultures were incubated in BACTEC 9120 (BD Diagnostics, Sparks, MD). Susceptibility of all bacterial isolates to antibiotics was tested using a disk-agar diffusion method and Clinical and Laboratory Standards Institute (CLSI) guidelines for interpretation of antibiotic susceptibility testing were used (CLSI, 2011).

Bacterial contaminants were excluded based on standard criteria (Weinstein *et al*, 1997; Weinstein, 2003). For each positive culture, type of isolated organism, time to culture positivity, date of specimen collection, biodata of patient on admission, and presence of focal infection and illness assessment were considered (Weinstein *et al*, 1997; Weinstein, 2003). Isolates were classified as true pathogens or contaminants as previously described (Murni *et al*, 2015). Only the results of true nosocomial pathogens were used for analysis.

Definition

Nosocomial bloodstream infections were defined using the United States Centers for Disease Control and Prevention (CDC) criteria. All isolates of a pathogen cultured from blood collected > 48 hours after hospitalization were considered as nosocomial bloodstream infection when there were corresponding clinical symptoms of infection and there was no contamination. If the same organism was found on repeat blood cultures collected within 14 days, the isolates were counted as one infection episode.

Culture-positive nosocomial bloodstream infection (BSI) must meet at least one of the following criteria: (1) the patient had an organism cultured from one or more blood cultures unrelated to another site of infection; or (2) the patient had at least one of the following clinical mani-

festations: fever (> 38.5°C), hypotension and positive laboratory results not related to another site of infection and common skin contaminant organisms [*ie*, diphtheroids (*Corynebacterium* spp), *Bacillus* (not *B. anthracis*) spp, *Propionibacterium* spp, coagulase-negative staphylococci (including *S. epidermidis*), viridans group streptococci, *Aerococcus* spp, and *Micrococcus* spp] were cultured from two or more blood cultures drawn on separate occasions; or (3) patients aged ≤ 1 year having at least one of the following signs or symptoms: fever (> 38.5°C), hypothermia (< 36.5°C), apnea, or bradycardia and positive laboratory results not related to another site of infection and common skin contaminant organisms [*ie*, diphtheroids (*Corynebacterium* spp), *Bacillus* (not *B. anthracis*) spp, *Propionibacterium* spp, coagulase-negative staphylococci (including *S. epidermidis*), viridans group streptococci, *Aerococcus* spp, and *Micrococcus* spp] were cultured from two or more blood cultures drawn on separate occasions (Horan *et al*, 2008).

Antibiotics used in antibiotic susceptibility testing were amikacin, ampicillin, ampicillin/sulbactam, cefepime, cefotaxime, cefpirome, cefuroxime, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fosfomycin, gentamicin, imipenem, meropenem, netilmycin, oxacillin, penicillin G, tetracycline, tobramycin, and vancomycin. Antibiotics prescribed at the pediatric ICU and pediatric wards, Dr Sardjito Hospital were amikacin, ampicillin, ampicillin/sulbactam, cefepime, cefotaxime, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fosfomycin, gentamicin, imipenem, meropenem, and vancomycin. Results of each nosocomial-isolated pathogen were reported as "susceptible" (S), "intermediate" (I), or "resistant" (R). Resistance is defined as being resistant to at least one of several antibiotics in the same class (Magiorakos *et al*, 2012; Sievert *et al*, 2013;Mathot *et al*, 2015). *Pseudomonas aeruginosa* resistant to extended-spectrum cephalosporins is defined when the isolate was reported as R or I to cefepime or ceftazidime. Enterobacteriaceae resistant to extended-spectrum cephalosporins was defined when the isolate was reported as R or I to cefepime, cefotaxime, ceftazidime, or ceftriaxone. Carbapenem resistance is defined when pathogens were reported as R or I to imipenem or meropenem. *P. aeruginosa* or *Escherichia coli* resistant to a fluoroquinolone is defined when these pathogens were reported as R or I to ciprofloxacin. *P. aeruginosa* resistant to aminoglycoside is defined when these pathogens were reported R or I to amikacin, gentamicin or tobramycin.

Non-MDR pathogen is defined as being resistant to at least one antibiotic in one class but less than 3 classes. Definition of MDR is based on a report of R or I for at least one of the antibiotics within an antimicrobial class, thus establishing non-susceptibility to that class and non-susceptibility to at least 3 of the specified classes for the given bacteria (Magiorakos *et al*, 2012; Sievert *et al*, 2013). In our setting, the definition of MDR *P. aeruginosa, Klebsiella pneumoniae, E. coli* and Enterobacteriaceae is resistance to 4 antibiotic classes: extended-spectrum cephalosporin, fluoroquinolone, aminoglycoside, and carbapenem. For *Acinetobacter baumanii* this includes 5 classes: extended-spectrum cephalosporin, fluoroquinolone, aminoglycoside, carbapenem, and ampicillin/sulbactam.

Outcome measures

We determined the proportion of patients with nosocomial bloodstream pathogens that were resistant to selected

antibiotics, the proportion with MDR infection, and the mortality among children with drug-sensitive, non-MDR, and MDR bacterial infections. We sought to understand the antibiotic pattern of commonly isolated pathogens in children with nosocomial bloodstream infection.

Data analysis

Patients with nosocomial bloodstream infections resistant to selected antibiotics and mortality related to antibiotic resistant organisms are presented as proportions. A $p < 0.05$ is considered statistically significant. The relative risk (RR) also was determined to compare mortality in patients with and without MDR nosocomial bloodstream infections.

RESULTS

One hundred and seventy-four microbiological isolates in the PICU and pediatric wards were cultured from blood samples from 170 patients with clinical features of nosocomial bloodstream infection (Table 1). Bacteria were identified in 168 cases and fungal species in 6 cases. Of the 168 nosocomial pathogenic bloodstream isolates, 148 (88%) were gram-negative bacteria and 20 (12%) were gram-positive bacteria (all of which were coagulase-negative Staphylococci).

P. aeruginosa was the most common 55% (93 isolates) gram-negative pathogen in nosocomial bloodstream infections. *Klebsiella* spp were found in 6% (10), Enterobacteriaceae in 4% (6), *A. baumanii* in 1% (2), and *E. coli* in < 1% (1) (Table 2). Detailed distribution of pathogens isolated in nosocomial bloodstream infections was shown in the previous published study (Murni *et al*, 2015).

The majority of pathogens were resistant to third generation cephalosporins, cefotaxime and ceftriaxone, but were sensitive to ceftazidime (Table 2). In general, nosocomial gram-negative pathogens had the highest susceptibility to amikacin, ciprofloxacin and imipenem, the latter observed in 145 (86%) isolates, followed by amikacin (142; 84%) and ciprofloxacin (141; 84%) (Table 1). Ceftazidime and chloramphenicol had comparable rates of *in vitro* sensitivity (75% and 74%, respectively).

High rates of non-MDR were noted. Sixty-two of 93 (67%) bloodstream infections caused by *P. aeruginosa* were resistant to one antibiotic in a given class but less than 3 classes (*ie*, non-MDR). Approximately 80% (8/10) of nosocomial bloodstream infection caused by *Klebsiella* spp were non-MDR, and 86% (6/7) of non-MDR nosocomial bloodstream infection were caused by Enterobacteriaceae including *E. coli* (Table 3). The antibiotic susceptibility patterns were similar between PICU and pediatric wards (data not shown).

Eleven patients (6%) developed infections caused by MDR organisms, of whom 7 died (Table 4). Among children with drug-susceptible bloodstream infection mortality was 25% (1/4) and in children with non-MDR blood stream infection mortality was 40% (62/155). Overall, MDR organisms causing nosocomial bloodstream infections were associated with increased risk of death with RR of 17.9 (95% CI: 5.2 - 61.8).

DISCUSSION

Nosocomial infections caused by antibiotic-resistant bacteria are difficult to treat and cause substantial mortality and morbidity, not to mention increase in the cost of treatment. Understanding the microbiology of nosocomial bloodstream infection is crucial to choose appropriate

Table 1
Baseline characteristics of patients.

Characteristics	Children with nosocomial bloodstream infection ($N = 170$) n (%)
Male	103 (61)
Age (months)	
≤ 12	73 (43)
> 12-60	47 (28)
> 60-120	21 (12)
> 120	29 (17)
Setting	
Pediatric ICU	87 (51)
Pediatric ward	83 (49)
Underlying disease	
Non-infectious disease	92 (54)
Infectious disease	44 (26)
Surgery	19 (11)
Malignancy	11 (6)
Sepsis	4 (2)
Malnutrition	12 (7)
Immunocompromised condition	14 (8)

ICU, intensive care unit.

antibiotic therapy and to further reduce or eliminate the emergence of antimicrobial resistance.

In this study, the most common causes of nosocomial bloodstream infections were gram-negative bacteria, in particular *P. aeruginosa*. This was similar to previous studies from Asia (Sritippayawan *et al*, 2009; Jean and Hsueh, 2011; Tseng *et al*, 2012; Ivady *et al*, 2016), but different from studies in Western countries where the most prevalent causes of nosocomial infections in developed countries are gram-positive bacteria (Sievert *et al*, 2013). There may be several reasons for this: (i) *P. aeruginosa* has minimal requirement for growth, and (ii) extensive use of antibiotics and inadequate compliance to infection control guidelines in such settings (Morrison and Wenzel, 1984). *P. aeruginosa* has an extracellular layer containing exopolysaccharides to protect the organism and to serve as an anti-phagocytic defense mechanism, thereby providing advantages over their bacteria competitors (Morrison and Wenzel, 1984). Moreover, *P. aeruginosa* can metabolize organic substrates, making this organism easy to survive and the organism can grow in various temperatures ranging from 20ºC to 42°C, with an optimal temperature of 37°C (Morrison and Wenzel, 1984). *P. aeruginosa* can survive in the environment in moist areas. Water supply in hospitals is likely to be an important source for *P. aeruginosa* colonization and infection, although transmission from contaminated faucets during hand washing process, instead of from within the water supply itself has been described (Navon-Venezia

Table 2
Antibiotic susceptibility test of nosocomial bloodstream pathogens.

Organism	Ciprofloxacin susceptibility	Ceftazidime susceptibility	Cefotaxime susceptibility	Ceftriaxone susceptibility	Chloramphenicol susceptibility	Gentamicin susceptibility	Amikacin susceptibility	Imipenem susceptibility
P. aeruginosa	84/93	82/93	17/93	20/93	74/93	41/93	79/93	85/93
K. pneumoniae	9/9	3/9	2/9	3/9	7/9	3/9	9/9	9/9
S. marcescens	9/11	6/11	0/11	0/11	10/11	10/11	11/11	11/11
B. cepacia	9/14	14/14	4/14	4/14	8/14	5/14	6/14	12/14
E. coli	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1
Enterobacter spp	5/6	4/6	1/6	1/6	1/6	1/6	6/6	6/6
Acinetobacter spp	4/7	5/7	1/7	2/7	4/7	3/7	4/7	3/7
Pseudomonas spp	6/6	4/6	0/6	1/6	5/6	4/6	6/6	6/6
Klebsiella spp	1/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1
CONS	13/19	6/19	5/19	4/19	-	-	18/19	10/19
Enterococcus spp	0/1	1/1	0/1	0/1	-	-	1/1	1/1
Total	141/168 (84%)	126/168 (75%)	31/168 (18%)	36/168 (21%)	110/148 (74%)	68/148 (46%)	142/168 (84%)	145/168 (86%)

CONS, coagulase-negative staphylococci.

et al, 2005). Therefore, hand hygiene might be very important to limit the transmission of *P. aeruginosa*.

Antibiotic resistance is a major public health problem in developing countries. Although > 90% of infections in our study were non-MDR but 6% were MDR, a proportion lower than a previous study (33.6%) (Ivady *et al*, 2016). This might be due to the previous study included patients with malignancy (33%) and this is significantly associated with MDR pathogens, whereas in our study the proportion of children with malignancy was only 6%.

Having a blood-stream infection with an MDR pathogen was associated with increased risk of death by a factor of 18. Although nosocomial infection is indeed one of several contributing factors that influences in-hospital death, this corresponded to results of previous study showing that the emergence of gram-negative resistance is associated with a 3-fold higher risk of death (Carmeli *et al*, 1999).

The proportion of MDR *P. aeruginosa* and *A. baumanii* was 9% and 100%, respectively, but *P. aeruginosa* was much more commonly isolated. These findings were comparable to a study in Thailand involving patients in PICU, but *A. baumanii* is the most common MDR pathogen causing nosocomial infection (50%) and *P. aeruginosa* is the second most common (16%) (Sritippayawan

Table 3
Proportion of patients with nosocomial bloodstream infection infected with pathogens resistant to certain antibiotics.

Organism resistant to antibiotics	No. of patients	No. of nosocomial BSI	No. resistant (%)
Pseudomonas aeruginosa	93	93	
Aminoglycosides			52 (56)
ESC			76 (82)
Fluoroquinolone			9 (10)
Carbapenem			8 (9)
MDR			8 (9)
Klebsiella	10	10	
Aminoglycosides			6 (60)
ESC			8 (80)
Carbapenem			-
MDR			-
Escherichia coli	1	1	
Aminoglycosides			1 (100)
ESC			-
Fluoroquinolone			-
Carbapenem			-
MDR			-
Enterobacteriaceae	6	6	
Aminoglycosides			5 (83)
ESC			5 (83)
Fluoroquinolone			1 (17)
Carbapenem			-
MDR			1 (17)
Acinetobacter baumanii	2	2	
Aminoglycosides			2 (100)
ESC			2 (100)
Fluoroquinolone			2 (100)
Carbapenem			2 (100)
Ampicillin/sulbactam			1 (50)
MDR			2 (100)

BSI, bloodstream infection. Aminoglycosides, resistance or intermediate to amikacin, gentamicin, or tobramycin; carbapenem, resistance or intermediate to imipenem; ESC, resistance or intermediate to extended spectrum cephalosporins (cefotaxime, ceftriaxone, ceftazidime, or cefepime); fluoroquinolone, resistance or intermediate to ciprofloxacine. MDR, multidrug resistance (resistant or intermediate resistant to at least one of the antibiotics within an antimicrobial class and at least 3 of the specified classes: for *P. aeruginosa, Klebsiella pneumoniae, E. coli* and Enterobacteriaceae, this included extended-spectrum cephalosporins, fluoroquinolones, aminoglycosides, and carbapenems; and for *Acinetobacter baumanii*, this included extended-spectrum cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, and ampicillin/sulbactam.

Table 4
Mortality in patients with antibiotic susceptible nosocomial BSI, mono-resistant nosocomial BSI, and MDR nosocomial BSI.

	Mortality (%)
Overall nosocomial BSI	70/170 (41)
Bacterial nosocomial BSI	70/168 (42)
Antibiotic susceptible nosocomial BSI	1/4 (25)
Non-MDR nosocomial BSI	62/153 (40)
BSI related to *P. aeruginosa*	37/85 (43)
BSI related to Enterobacteriaceae	2/5 (40)
BSI related to *Klebsiella*	5/10 (50)
BSI related to *E. coli*	1/1 (100)
MDR nosocomial BSI	7/11 (64)
BSI related to MDR *P. aeruginosa*	4/8 (50)
BSI related to MDR Enterobacteriaceae	1/1 (100)
BSI related to MDR *A. baumanii*	2/2 (100)

BSI, bloodstream infection; MDR, multidrug resistant.

et al, 2009). The occurrence of MDR *P. aeruginosa* in our study was in agreement with a previous surveillance study conducted in a developed country reporting a prevalence of 1% to 16% (D'Agata, 2004). However, our resistance findings were much higher compared to SENTRY antimicrobial surveillance survey in the Asia-Pacific region, which reported a proportion of MDR *P. aeruginosa* of 1.6% (Gales *et al*, 2001; Obritsch *et al*, 2005). Differences among countries, such as demography, facilities, healthcare environment, number of health workers, and infectious disease burden, may result in prevalence of antimicrobial resistance of nosocomial infection different from this study.

Our study demonstrates a high burden of antibiotic resistant gram-negative bacteria causing nosocomial infection and MDR bacteria contributing to a significant increase in mortality. These findings should alert health workers and managers to the need for surveillance and implementation of infection control interventions. Containment of antibiotic resistance in hospitals requires an integrated multi-faceted approach given the high use of antibiotics in these settings (WHO, 2001). Such a program needs effective infection control and antibiotic stewardship. The latter should provide guidelines for antibiotic prescribing practices, conduct routine surveillance and audit of infection control and antibiotic prescribing practices, apply isolation and universal or standard precautions, and institute cleaning and good hand hygiene practice to prevent cross-transmission (WHO, 2001; Murni *et al*, 2015). Strict compliance to good hand hygiene practice, judicious use of antibiotics for treating nosocomial infections, limiting invasive procedures, and timely discharge from pediatric ICU can limit the appearance and spread of antibiotic resistance (Toltzis and Blumer, 2001). These measures are similarly applied in our hospital. However, the effectiveness of routine surveillance to identify patients colonized with MDR

gram-negative pathogens and initiation of isolation precaution for patients colonized or infected with MDR gram-negative pathogens are still uncertain (Harris *et al*, 2006).

Antibiotics given as monotherapy that provided the highest efficacy against nosocomial bloodstream pathogens were amikacin, ceftazidime, ciprofloxacin, and imipenem. Therefore, the use of those antibiotics should be restricted for hospital-acquired infections only, and not used for community-acquired infections. Furthermore, use of imipenem should be reserved for isolates of proven resistance to other antibiotics as resistance to imipenem is readily developed in *P. aeruginosa*, with a hazard ratio of 2.8 compared to 0.7 for ceftazidime and 0.8 for ciprofloxacin (Carmeli *et al*, 1999).

Whether to use combination or single antibiotic for nosocomial bloodstream infections is debatable. Combination of two synergistic antibiotics *in vitro* may enhance clinical efficacy and prevent emergence of resistant pathogens, but the drug combination therapy is organism- and infection-site specific (Navon-Venezia *et al*, 2005). In one study mortality in *P. aeruginosa* infection decreases by 50% when using a combination of antibiotics with an RR of 0.50 (95% CI: 0.32 - 0.79), and a combination of a beta-lactamase inhibitor and a quinolone produces better outcome compared to a combination of a beta-lactamase inhibitor and an aminoglycoside (Safdar *et al*, 2004). However, a meta-analysis found no benefit on mortality and prevention of antimicrobial resistance between using combined antibiotics compared to a single antibiotic (Paul *et al*, 2004).

The strength of our study is that we determined clinical infection, not colonization or contamination, when performing nosocomial pathogen surveillance. We also standardized the definition of non-MDR and MDR, allowing comparison with other studies. However, we did not use molecular techniques to determine the exact mechanisms of antimicrobial resistance. The ability to generalize the results from one hospital may be limited, but many hospitals in Indonesia and developing countries in the Asia-Pacific region have very limited microbiological facilities, so this study should help to shine a light on the problem of hospital-acquired and non-MDR and MDR bacterial infections, and their clinical consequences.

In conclusion, this study demonstrates that there was a substantial burden of morbidity and mortality from nosocomial bloodstream infections caused by resistant bacteria in children in a teaching hospital in Indonesia. Use of amikacin, carbapenems, ceftazidime, or ciprofloxacin should be preserved for the specific treatment of nosocomial infections. Antibiotic stewardship and good hand hygiene practice are the corner stones for controlling nosocomial pathogens and antibiotic resistance.

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