A 5-YEARS REVIEW OF ANTIBIOTIC RESISTANCE OF ORGANISM CULTURED FROM PATIENTS IN HUSM NICU COMPARED TO OTHER ICUs

by

DR NOORZAITUN ARIFFIN

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ABBREVIATIONS

NICU	neonatal intensive care units
ICU/HDU	intensive care units /high dependency units
HUSM	Hospital Universiti Sains Malaysia
USA	United States America
ELBW	Extremely-Low-Birth-Weight
MDRGN	Multiple Drug Resistant Gram Negative Bacilli
MRSA	Methicillin Resistant *Staphylococcus Aureus*
VRE	Vancomycin Resistant *Enterococci*
DNA	Deoxyribos Nucleic Acid
ESBL	Extended Spectrum β-Lactamases
PFGE	Pulsed-Field Gel Electrophoresis
1 NILAM	Neonatal Intensive Care Unit, HUSM
CME	Continuous Medical Education
CNE	Continuous Nurse Education
CLSI	Clinical and Laboratory Standards Institute
DOA	Date of admission
SPSS	Statistical Package for the Social Sciences

ABSTRACT

Most of the infections in intensive care settings need to be treated empirically before the causative organism is known. The right choice of antibiotics is very important and can make the difference between life and death of the infected patient. On the other hand the overuse of broad spectrum antibiotics can lead to resistance. It is very important to know the resistance pattern in particular wards in order to make rational decisions for empirical therapy

Aim

To determine and compare the bacterial demographics and antibiotic resistance pattern of bacteria in the neonatal intensive care unit (NICU) and ICU/HD (intensive care units /high dependency units) in HUSM (Hospital Universiti Sains Malaysia)

Methods

This study is a retrospective descriptive study of organisms cultured from the NICU, HUSM, and their antibiotic resistance pattern over the last 5 years (2005-2009) compared to organisms (and their resistance pattern) grown from other intensive or high dependency care units in the same hospital during the same period. The file containing the positive cultures from each ward in HUSM was available in the

archives of the Microbiology Laboratory of HUSM, Kubang Kerian. These data were cleaned base on admission date and antibiotic resistance and they were subsequently analyzed statistically using SPSS version 12.1.

Results

There were 6403 bacteria included in the study: 1320 from the NICU and 5083 from ICU/HDU. In the NICU 25.8% were cultured from early specimens and 74.2% from late specimens. In the ICU/HDU, 43.0% came from early and 57.0% from late specimens. In the ICU/HDU Gram negative bacteria were significantly more common than in the NICU. (60% vs. 49% , $p < 0.001$). The most common Gram negative organism cultured from the ICU/HDU was *Acinetobacter* spp. The most common one in the NICU was *Klebsiella pneumoniae* ss pneumonia. Among the common Gram negative organisms there were significant differences between the ICU/HDU and NICU. The most common Gram positive organism cultured from the ICU/HDU was *Staphylococcus aureus (*40.0 vs. 18.7%; $p<0.001$) and from the NICU was coagulase-negative staphylococci. (32.2 vs. 62.3%; $p<0.001$).

Regarding antibiotic resistance, it was significantly lower in the NICU, compared to other ICU/HDU. The most pronounced differences were found for Gram negative organisms cultured but also for Gram positive organisms there was a less pronounced lower resistance in the NICU.

Conclusion

This study shows that there are marked differences between the demographics and antibiotic resistance pattern between the NICU and other intensive care areas of HUSM.

ABSTRAK

Bahasa Malaysia

Pengenalan

Kebanyakan jangkitan kuman di unit rawatan rapi perlu di rawat secara *empirikal* sebelum organisma penyebab jangkitan diketahui. Pilihan antibiotik yang tepat adalah sangat penting demi untuk keselamatan pesakit yang dirawat. Walau bagaimana pun, penggunaan antibiotik *"broad spectrum"* secara tidak terkawal boleh menyebabkan *"resistance"*. Adalah penting untuk mengetahui corak *"resistance"* di dalam wad-wad tertentu untuk membuat keputusan yang rasional untuk member rawatan secara *empirikal.*

Tujuan

Untuk mengenalpasti dan membandingkan populasi kuman dan kadar ketidakkeberkesanan antibiotik di unit rawatan rapi bayi dan unit rawatan rapi dewasa di HUSM.

Kaedah

Kajian *'retrospective'* telah dijalankan pada keputusan *'specimen culture'* dari unit rawatan rapi bayi, HUSM dan kadar keberkesanan antibiotik untuk 5 tahun yang lepas (2005-2009) dibandingkan dengan lain- lain unit rawatan rapi. Maklumat keputusan *'specimen culture'* diperolehi dari arkib makmal kajikuman HUSM, Kubang Kerian. Data yang diperolehi disaring berpandukan tarikh kemasukan ke wad dan kadar ketidakkeberkesanan antibiotik dan seterusnya di analisis dengan SPSS versi 12.1.

Keputusan

Selepas saringan 6403 keputusan *'specimen culture'* yang menepati kehendak kajian: 1320 dari unit rawatan rapi bayi dan 5083 dari unit rawatan rapi dewasa. Dari unit rawatan bayi 25.8% adalah jangkitan awal kuman dan 74.2% adalah jangkitan kuman lewat. Dari unit rawatan rapi dewasa 43.0% adalah jangkitan kuman awal dan 57.0% adalah dari jangkitan kuman lewat. Di Unit rawatan rapi dewasa kuman *'Gram negative'* lebih banyak dibandingkan dengan unit rawatan bayi. (60% vs. 49%, $p < 0.001$). kuman *'Gram negative'* yang paling banyak dikesan di unit rawatan rapi dewasa adalah *Acinetobacter* spp. dan *Klebsiella pneumoniae* ss pneumonia. di unit rawatan rapi bayi. Di antara kuman *'Gram negative'* yang biasa ditemui ini menunjukkan perbezaan yang penting dari segi statistiknya. Di antara kuman *'Gram Positive'* yang paling banyak di temui di unit rawatan rapi dewasa adalah *Staphylococcus aureus* (40.0 vs.18.7%; $p<0.001$) dan coagulase-negative staphylococci. (32.2 vs. 62.3%; $p<0.001$) di unit rawatan rapi bayi. Dari segi ketidakkebersanan antibiotik, kadar nya di unit rawatan rapi bayi adalah lebih rendah berbanding unit rawatan rapi dewasa. Perbezaan yang paling ketara adalah pada

kuman *'Gram negative'* bila dibandingkan kedua-dua unit ini dan perbezaan kadar ketidakkebersanan antibiotik pada kuman *'Gram positive* adalah tidak begitu ketara.

Kesimpulan

Kajian ini menunjukan perbezaan yang ketara dari segi taburan kuman dan kadar ketidakkeberkesanan antibiotik antara wad rawatan bayi dan dewasa.

INTRODUCTION

1. Introduction

1.1. Background of the study

Infections are a major problem in intensive care units (ICU) and in high dependency units (HDU). Classically infections in an ICU or HDU are divided into early onset infections which are generally believed to be acquired before the patient entered that ward and late onset, which are generally believed to be acquired after the patient entered that ward (nosocomial infection).

Most of these infections need to be treated empirically before the causative organism is known. The right choice of antibiotics is very important and can make the difference between life and death of the infected patient. This creates a tendency among clinicians to use for each individual patient the broadest spectrum antibiotics as an empirical therapy. Overuse of broad spectrum antibiotics will lead to resistance of the bacteria in the ICU or HDU towards the antibiotics used.(Ariffin, Navaratnam et al. 2004).

Rational antibiotic use is very important and involves a thorough knowledge of the resistance pattern of bacteria that are present in the specific ward. Reviews of antibiotic resistance play a crucial role in rational antibiotic use.

Other important factors playing a role in the development of resistance of ICU or HDU bacteria include the effective implementation of infection control measures which limit the cross contamination of patients sharing the same ward.

Within Hospital Universiti Sains Malaysia (HUSM) the neonatal intensive care unit (NICU) has between 1800 to 2100 admissions per year. Within the five last years a strict antibiotic policy has been followed and infection control measures have been tightly implemented, including a written hand washing policy and implementation of various teaching modules regarding infectious control during continuous medical education for nurses and doctors in the ward. Upon review of the monthly reports received from the Infectious Control Unit of the hospital, it appeared that the type of organisms cultured and the antibiotic resistance differed quite substantially between the NICU and the other ICUs and HDUs within HUSM.

This study was undertaken to determine the type of organisms that were cultured from patients admitted to the NICU over the past 5 years and to compare them with those cultured from patients admitted to other ICUs and HDUs. A second important objective was to compare antibiotic resistance of the organisms between the NICU and the other ICU/HDUs. The availability of these data will allow the hospital Infection Control Unit to find causes for potentially existing differences and plan appropriate actions, where needed.

In the following literature review, the importance of infections in intensive care and high dependency areas and the resistance pattern of commonly isolated organisms will be discussed. This will be followed by an overview of studies that have compared bacterial resistance among intensive care areas inter and intra hospital.

1.2 Literature review

1.2.1 Infections in intensive care and high dependency areas

1.2.1.1 Overview

As stated in the background of this study, the infections in the intensive care areas tend to be divided in early and late onset infections. The cut off limit between early and late varies greatly among studies but 48 hours (Gurskis, Asembergiene et al. 2009) after admission is quite often accepted as a reasonable cut off limit. The microbiology and bacterial resistance of both types of infections tend to be different and they may require different policies for rational antibiotic use.

1.2.1.2 Early onset infections

Early onset infections are most often community acquired infections, unless the patients have recently spent time in the hospital. These community acquired infections are often caused by bacteria that have not yet, or only to a limited extend been exposed to broad spectrum antibiotics. The bacteria causing community acquired infections in paediatric or adult wards are expected to be different from those early onset infections in the NICU which consist mainly of congenital infections, for which the causative organisms come predominantly from the maternal genital tract. These organisms are typically group B streptococci or gram negative bacteria that are quite sensitive to antibiotics (Tiskumara *et al.*, 2009). Recent

reports however from the USA and India (Stoll, Hansen et al. 2005; Bizzarro, Dembry et al. 2008; Sundaram, Kumar et al. 2009) have indicated that in several places the differences in bacteria and resistance to antibiotics between early onset infections in the NICU and late onset infections is becoming less obvious or even non-existent.

1.2.1.3 Late onset or nosocomial infections

Nosocomial infections in neonatal intensive care units (NICUs) are an important problem. Environmental and host factors play an important role in the high rate of infection, reported by many NICUs. Because of the innate immuno-compromised state of sick newborns, reported infection rates tend to be higher in NICUs compared to paediatric and adult ICUs. (Gaynes *et al.*, 1996, (Gaynes, Edwards et al. 1996; Stoll, Hansen et al. 2002; Baltimore 2003).

Since the 1980s, NICUs provide more and more frequently care for high risk infants such as extremely-low-birth-weight (ELBW) and chronically ill infants. The need of prolonged use of ventilatory support as well as prolonged hospital stay increases the chance for late onset nosocomial infection (Baltimore 2003).

Infections with gram negative organisms were associated with multiple drug resistance toward commonly used drugs in NICUs. (D'Agata, Venkataraman et al. 1999; Harbarth, Sudre et al. 1999; Almuneef, Baltimore et al. 2001; Toltzis, Dul et al. 2001; Bisson, Fishman et al. 2002; Pessoa-Silva, Meurer Moreira et al. 2003; Waters, Larson et al. 2004). The intestines of hospitalized infants are the main

reservoir for multiple drug resistant gram negative bacilli (MDRGN) (Donskey 2004).

Preferential growth of MDRGN (mainly through the extensive use of broad spectrum antibiotics) and their dissemination, (mainly due to cross transmission via hands of care givers, contaminated equipments or in animated objects, and failure of other infection control practices) are responsible for a diffuse horizontal spread of infection (D'Agata, Venkataraman et al. 1999; Harbarth, Sudre et al. 1999; Almuneef, Baltimore et al. 2001; Bisson, Fishman et al. 2002; Pessoa-Silva, Meurer Moreira et al. 2003; Waters, Larson et al. 2004). The incidence of infection caused by β-lactam resistant organisms is reported to be increased and is usually associated with clonal infection outbreaks (D'Agata, Venkataraman et al. 1999; Bisson, Fishman et al. 2002).

Several attempts have been made to determine the epidemiology of nosocomial MDRGN by monitoring risk factors and by using molecular typing studies to trace the horizontal transmission between newborn (D'Agata, Venkataraman et al. 1999; Almuneef, Baltimore et al. 2001; Toltzis, Dul et al. 2001; Pessoa-Silva, Meurer Moreira et al. 2003; Waters, Larson et al. 2004). However during endemic periods, the dynamics of transmission of MDRGN are expected to be variable, depending on the interaction of human and environmental ecology of NICUs. Indeed surveillance studies have shown quite a variety of findings: Drug resistant bacilli identified through clonal technique via molecular analysis accounted for between 12% and more than 50% of isolates from colonized patients (Harbarth,

Sudre et al. 1999; Toltzis, Dul et al. 2001; Pessoa-Silva, Meurer Moreira et al. 2003; Waters, Larson et al. 2004)

1.2.1.4 Resistance.

The ICU related resistant organisms have raised awareness for past decades, especially *methicillin resistant Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE) and MDRGN.

There is a lot of evidence from adult patients that these organisms prolong hospital stay and increase the health costs and that they are associated with high mortality. Mortality is increased partially due to the limited antibiotic choice in the presence of resistance and forced use of antibiotics that have poor tissue penetration (for example – vancomycin for MRSA) or that are bacteriostatic rather than cidal (for example – trimethoprim-sulfamethoxasole for multiple drug resistant *Stenotropho-monas*). In addition, for many patients, the antibiotic resistance may cause delay in the prescription of effective antibiotics for 2 to 3 days due to time required to complete the drug susceptibility testing.

In the NICU an additional consequence of increased bacterial resistance to antibiotics is that the clinicians are forced to use antibiotics for which there are few or no data regarding pharmacokinetic, distribution or toxicity in premature infants. Given the alarming consequences of infection by antibiotic resistant organisms it is imperative to understand and apply strategies to contain their spread particularly among the patients with a deficient immune status.

The number of preterm infants requiring intensive therapies and being admitted to NICU is increasing. Early use of empiric broad spectrum antibiotics is a strategy that will select resistant bacteria.

Empirical antibiotic regimes used in suspected septicemia in neonates need to be able to cover the group B streptococcus, *Listeria* spp. *and Escherichia coli.* Broad spectrum penicillin such as ampicillin or amoxicillin combined with an aminoglycoside is a commonly practiced regime.(Tiskumara, Fakharee et al. 2009).

Third generation cephalosporins have been shown by many reports to select strains of *Enterobacter* spp *and Serattia* spp which contain chromosomal β-lactamase genes. The use of third generation cephalosporins leads to production of plasmids carrying DNA for extended spectrum β lactemases (ESBL)(Singh, Patel et al. 2002).

Since antibiotic resistance among Gram-negative bacilli involve multiple resistance phenotypes, the mechanism responsible for resistance is multiple too. Enzymatic antibiotic inactivation is the most prominent bacterial resistance mechanism among this group of organism.

Hydrolysis of antibiotic β-lactam bond by β-lactamase is the most common enzymatic antibiotic inactivation. A large group of β-lactamases has been identified in Gram negative organisms; with almost monthly additional enzymes have been reported (Bush, Jacoby et al. 1995; Heritage, M'Zali et al. 1999) and most new

enzymes formed represent single amino acid substitution of previously characterized enzymes that develop under antibiotic pressure (Heritage, M'Zali et al. 1999) for example the extended spectrum β-lactamases (ESBL).

i) Primary plasmid–borne enzymes have changed to hydrolyze greater numbers of advanced-generation β-lactam antibiotics. Many β -lactamase enzymes, particularly the chromosomal ampC β -lactamases are substrate inducible, so the organisms which were previously sensitive to a β -lactam antibiotic may become highly resistant during therapy.

ii) A second group of important inactivating enzymes produced by Gram negative bacteria conjugates aminoglycosides through phosphorilation, adenylation and acetylation. All of aminoglycosides that are commonly used are susceptible to be inactivated by one or more of these enzymes.

B-lactamase-mediated resistance is almost always found together with other mechanisms of resistance; this leads to a single Gram negative organism that may be resistant towards multiple commonly used antibiotics.

In addition to the production of inactivating enzymes, there are two other mechanisms for resistance among Gram negative bacteria:

i) Prevention of accumulation of antibiotics in the bacterial cell may be partially present by the hydrophobic outer membrane properties of gram negative bacteria. Other than that, aqueous diffusion channels, porins, on

the surface of bacteria, may be altered under certain pressure to become impermeable to some antibiotics. Sometimes this mechanism works together with others; for example: porin mutation with β-lactamase production can result in very marked resistance to almost all the β-lactam antibiotics in selected isolates of *Pseudomonas and Klebsiella* (Martinez-Martinez, Pascual et al. 1999). Some Gram negative bacteria express efflux pump properties to actively remove the antibiotic from the intracellular area. This mechanism is commonly seen in fluoroquinolone resistance (Nikaido 1998).

ii) Biochemical alteration of the antibiotic's cellular target leading to rapid development of resistance of Gram negative bacteria toward the fluoroquinolone group of antibiotics. It is through single or multiple amino acid substitutions on DNA gyrase or topoisomerase IV (Lang, De Fina et al. 2001).

1.2.1.5 Overview of studies comparing antibiotic resistance between wards

There are several reported studies that compare antibiotic resistance among wards. Without aiming to give a complete overview some of the more relevant among these studies are briefly discussed below.

In a study conducted between May 2001 and November 2003, in a burn unit ICU, *Pseudomonas aeruginosa* was the most frequent pathogen isolated (40.4%), followed by *Staphylococcus aureus* (29.3%) and *Acinobacter* spp. (9.8%). The antibiotics most effective against *Pseudomonas aeruginosa* in the ICU Burn Unit

were piperacillin-tazobactam and sulbactam-cefoperazon. The bacteriological profile and antibiotic resistance pattern were significantly different in other intensive care units within the same hospital (Yildirim, Nursal et al. 2005). This knowledge is crucial for early treatment of infections in burned patients

A study was done in Turkey covering four years of antibiotic sensitivity rates of *Pseudomonas aeruginosa* and *Acinobacter baumannii* strains isolated from patients in ICU and step down units from the year 2003 until 2006. The most sensitive antibiotic for *Pseudomonas aeruginosa* in 2003 was pipracillin-tazobactam (84%) followed by ciprofloxacin (79%), imipenem (77.5%) and meropenem (69%). Decreasing sensitivity was noted in 2006 towards similar antibiotics: 67%, 72%, 51% and 45% respectively. It was noted in this study that the ICU can act as the reservoir of resistant strains of bacteria which then spread to step-down units. The authors concluded that there was an urgent need to control the antibiotics used. (Aliskan, Colakoglu et al. 2008)

A survey done in the Department of Burns and Plastic Surgery, at Weifang People's Hospital, China evaluated the distribution of pathogens and their antibiotic resistance in order to provide the reference for antibiotic policies in clinical practice. The survey was a retrospective analysis from 2001 until 2006. It involved the cultures from wounds, blood, venous catheters, sputum, urine and purulent discharges. The distribution of pathogens was: Gram negative bacteria (52.6%), Gram positive bacteria (40.5%) and fungal (6.9%). The main pathogens were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinobacter* species and *Escherichia coli*. The MRSA in this study were 100% resistance to levofloxacilin,