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ORIGINAL ARTICLE

The Clinical Outcome and Antibiotic Sensitivity Pattern of Enterobacter Spp. Culture Positive Neonates Admitted at Cebu Doctors' University Hospital – Neonatal Intensive Care Unit (2005-2008)

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ABSTRACT

Introduction: Enterobacter spp., a gram negative organism, is an important nosocomial pathogen. It is capable of developing resistance during ß-lactam therapy by expressing genes that encode for Extended-Spectrum ß-lactamases (ESBLs).

Objectives: This aim of this research is to determine the clinical outcome of neonates with Enterobacter spp. positive blood culture and the antibiotic sensitivity pattern of these isolates at Cebu Doctors' University Hospital Neonatal Intensive Care Unit.

Methods: This descriptive, cross-sectional, retrospective study retrieved the list of neonates admitted at Cebu Doctors' Hospital –Neonatal Intensive Care Unit (CDUH-NICU) from January 2005 to December 2008 whose bloods were taken for culture. The antibiotic sensitivity patterns of the Enterobacter positive cases were reviewed, along with broad-spectrum cephalosporin (BSC) resistant and multiresistant Enterobacter spp. (MRE) determination and clinical outcome. The relationship between outcome and MRE was analyzed using the Pearson Chi-square test.

Results: Out of 1312 samples, only 110 (8.4%) had positive bacterial isolates. Twenty-five grew Enterobacter spp. The overall mortality rate among the neonates with Enterobacter spp. was 56%. It was statistically significant ($p<0.013$). The organism was most sensitive to Imipenem (100%), followed by Meropenem (92%), and then Cefepime and Piperacillin-Tazobactam (80%). Of the 25 isolates, 60% were BSC resistant, 16% non-BSC resistant, and 24% were MRE. For the six neonates with MRE, the mortality rate was 50% and survival rate was 33.3%. One patient with MRE was brought home against medical advice. The mortality rate among MRE cases was not statistically significant.

Conclusion: Most isolates were BSC-resistant while 24% were MRE. Enterobacter was most sensitive to Imipenem, Meropenem, Cefepime, and Piperacillin-Tazobactam.

INTRODUCTION

Infections in neonatal intensive care units have been a recognized cause for concern for many years. According to World Health Organization (WHO) estimates, neonatal sepsis remains the major cause of mortality among the five million neonatal deaths per year.[1]

The spectrum of organisms that cause neonatal sepsis changes over time and varies from region to region. Group B streptococcal disease is the most important cause of neonatal sepsis in Europe and North America[2,3] while there is a preponderance of gram-negative organisms in tropical and developing countries.[4,5]

Enterobacter spp. is a gram-negative organism and is an important nosocomial pathogen. It can cause significant morbidity and mortality and can complicate infection management because of its resistance to multiple antibiotics. Such an organism is capable of developing resistance during ß-lactam therapy by expressing genes that encode Extended-Spectrum ß-lactamases (ESBLs),[6] which makes it very hard to kill compared to other gram negative organisms. Overall, Enterobacter spp. has emerged as an important pathogen in neonatal units, with several outbreaks of infection being reported.[7]

Part of Cebu Doctor's University Hospital's reinforcement of good hygiene practices is the active regular program of surveillance done on culture growth by the Infectious Control Committee. According to the data gathered from the committee from January 2007 to December 2008, about 14 out of 29 (48%) positive blood cultures taken from neonates admitted at CDUH-NICU were Enterobacter spp.[12] Given this high rate, the researcher aimed to study the antibiotic sensitivity pattern and clinical outcome of neonates with Enterobacter positive blood culture at the Cebu Doctors' University Hospital–Neonatal Intensive Care Unit and is hopeful that the results of this study will provide a useful guide in determining the antibiotic treatment options for CDUH–NICU patients affected by this organism.

MATERIALS AND METHODS

Study design

In-patient microbiology section's logbooks were retrieved to get the list of neonates admitted at CDUH-NICU whose bloods were taken for culture (non-repetitive blood culture).

The researcher noted the total number of blood cultures which were positive for organisms; but, only the neonates who grew Enterobacter species on the blood culture from January 2005 to December 2008 had their medical charts at the Medical Records Section retrieved for antibiotic sensitivity pattern, broad-spectrum cephalosporin (BSC) resistant and multi resistant Enterobacter spp. (MRE) determination and clinical outcome (died or survived). For data analysis, the researcher used Pearson Chi-square in determining the relationship between clinical outcome and MRE.

Microbiological Methods

Blood samples were processed by the Bactec NR-730 system (Becton–Dickinson Microbiology System) and routinely maintained for seven days. Isolates were identified according to standard techniques. Antibiotic susceptibility was determined by the disc diffusion method, which is in accordance to the recommendations of the Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines.14

Antibiotic susceptibility testing of Enterobacter spp. isolates was reviewed. There were 29 antibiotic discs used and these were the following:

Cefazolin, Cephalothin, Cefuroxime, Cefepime, Cefoperazone, Cefoxitin, Cefotaxime, Ceftriaxone, Ceftazidime, Cefixime, Piperacillin-Tazobactam, Ampicillin-Sulbactam, Ticarcillin-Clavulanate, Meropenem, Ertapenem, Imipenem, Ciprofloxacin, Levofloxacin, Ofloxacin, Ampicillin, Amikacin, Gentamicin, Co-Amoxiclav, Trimethoprim-Sulfamethoxazole (TMP-SMZ), Aztreonam, Netilmycin, Tobramycin, Minocycline and Chloramphenicol

DEFINITION OF TERMS[13]

Bacteremia – is the presence of viable bacteria in the circulating bloodstream. This is confirmed by a positive blood culture.

Neonatal Sepsis – is a clinical syndrome characterized by systemic signs of infection and accompanied by bacteremias in the first month of life.

Nosocomial Infection in neonates – is an infection that develops after 48 hours from delivery that may be localized or systemic, and is due to an infectious agent or its toxin, that may or may not manifest during the infant's hospital stay.

Broad Spectrum Cephalosporin (BSC) resistance- in vitro resistance to Cefotaxime or Ceftazidime.

Multiresistant Enterobacter (MRE)- a strain with in vitro resistance to BSC, Ciprofloxacin and Gentamicin. In determining MRE, intermediate susceptibility to each antibiotic was considered as resistance.[15]

RESULTS

During the four-year study period, a total of 1,312 repetitive blood cultures were taken; one hundred ten had positive bacterial growth (110/1312 or 8.4%). Of the 110 positive blood cultures, 25 (22.7%) were Enterobacter spp., of which the following were isolated: Enterobacter cloacae, 11 and Enterobacter aerogenes, 14. There was evidence of clustering of cases of Enterobacter spp. 16/25(76.2%) that suggested an outbreak in the year 2008. As shown in Figure 2, the incidence of Enterobacter spp. has risen over the last four years with P value of <0.002 which is statistically significant.

The bacterial pathogens isolated from the 110 positive blood cultures are illustrated in Figure 2 and Table 1. The most common pathogen isolated was Staphylococcus spp., comprising of 8 (40%), 14 (40%), and 10 (29.4%) in the years 2005, 2006, and 2007, respectively. This was followed by Klebsiella pneumoniae and Streptococcus spp. with 3 (15%), 8 (22.9%) and 3 (15%), 5 (14.3%) in the years 2005 and

Figure1. Study design algorithm.

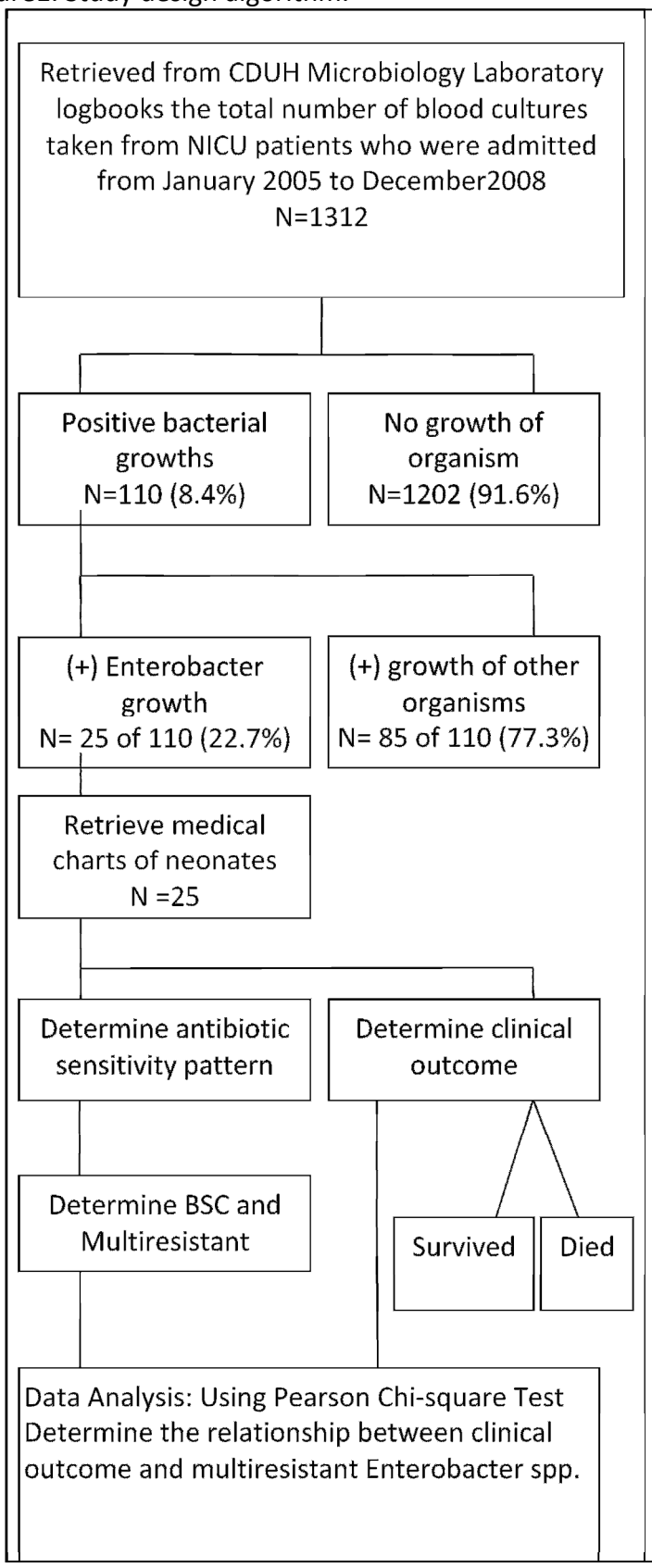
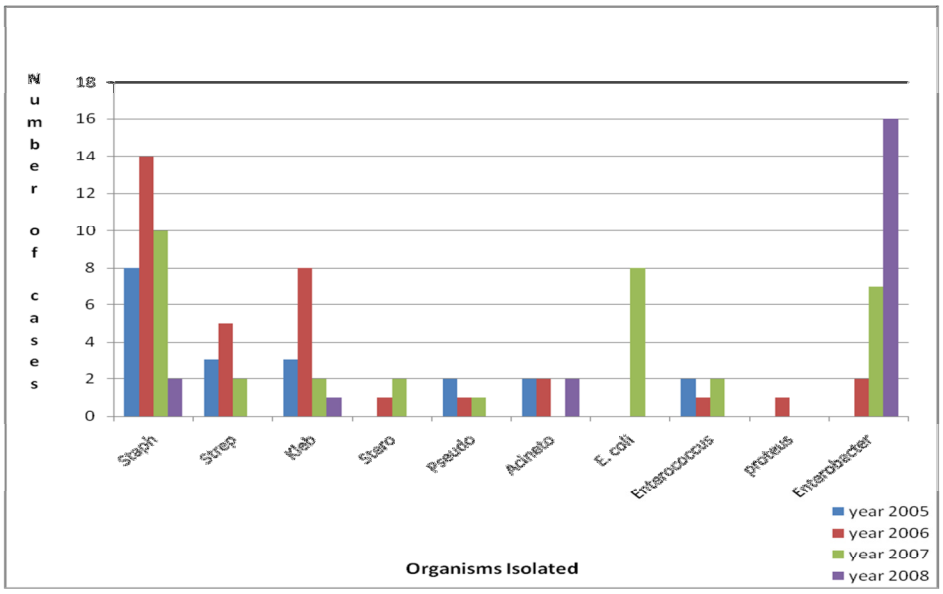


Figure 2. Organisms isolated from the blood culture of neonates admitted at CDUH -NICU from January 2005 to December 2008.



(Staph – Staphylococcus spp. , Strep – Streptococcus spp., Kleb – Klebsiella spp., Stero – Stenotrophomonas spp., Pseudo – Pseudomonas aeruginosa, Acineto – Acinetobacter baumanii, Enterococcus – Enterococcus spp., Proteus – Proteus mirabilis, Enterobacter – Enterobacter spp.)

Figure 3. Antibiotic sensitivity pattern of Enterobacter spp. grown from the Blood culture of CDUH-NICU patients from January 2005 to December 2008.

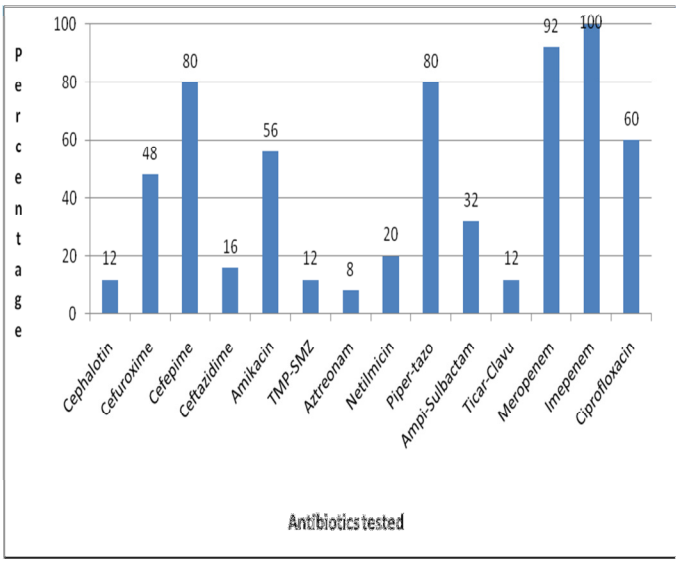
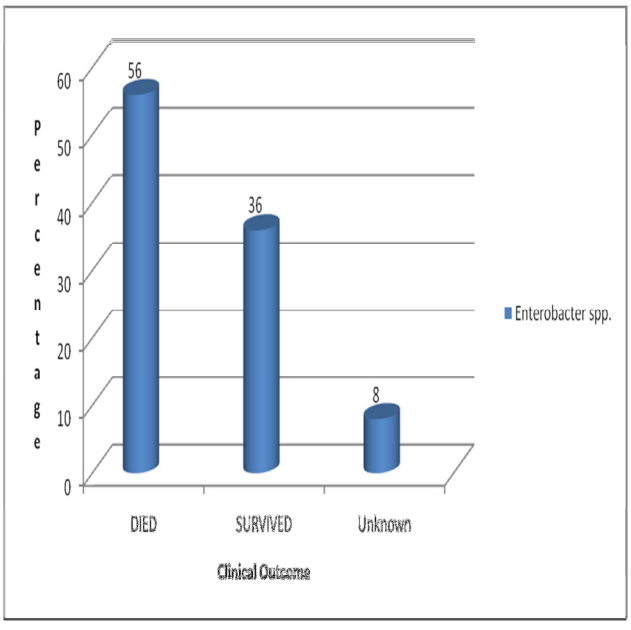


Figure 4 Clinical outcomes of CDUH-NICU patients with Enterobacter spp. from January 2005 to December 2008. (P value <0.013)



2006, respectively. However, on year 2007, the 2nd most common pathogen isolated was Escherichia coli with 8 (23.5%) and in the 2008, Enterobacter spp. was the most common pathogen isolated at 16 (76.2%); Staphylococcus spp. came only second with 2 (9.5%).

For the susceptibility testing of Enterobacter spp. positive cultures it was noted that out of the 29 listed antibiotics, only 14 antibiotic discs were tested on the 25 bacterial isolates. These antibiotics were Cephalothin, Cefuroxime, Cefepime, Ceftazidime, Amikacin, TMZ-SMZ, Aztreonam, Netilmycin, Piperacillin-Tazobactam, Ampicillin-Sulbactam, Ticarcillin-Clavulanate, Meropenem, Imipenem, and Ciprofloxacin. Fifteen of the antibiotic discs were unavailable at the laboratory at some point in time and were not used for testing.

All Enterobacter spp. positive cultures were most sensitive to Imipenem at 100%. It was followed by Meropenem at 92%, Cefepime and Piperacillin-Tazobactam at 80%. It is most resistant to Trimethoprim-Sulfamethoxazole and Aztreonam at 88%, followed by Cephalothin at 84%, Ceftazidime at 84% and Ticarcillin-Clavulanate at 72%. The rest of the rates of antibiotics' sensitivity are seen in Figures 3.

Among the 25 neonates with Enterobacter spp., 2/25 (8%) went home against medical advice; their statuses remain unknown. Of the 23 neonates who stayed for treatment, 14/25 (56%) died and 9/25 (36%) survived. As shown in Figure 4, the mortality rate of neonates with Enterobacter spp. is statistically significant with a P value of <0.013.

Of the 25 isolates, 15/25 (60%) were BSC resistant, 4/25 (16%) non-BSC resistant, and 6/25 (24%) were MRE. For the six neonates with MRE, the mortality rate was 3/6 (50%) and survival rate was 2/6 (33.3%). One patient with MRE was brought home against medical advice. The mortality rate among MRE cases was not statistically significant. Table 2 shows the tabulated clinical outcome, BSC resistant and MRE pattern.

Table 2. The Clinical Outcomes, BSC resistant and MRE pattern of Enterobacter Positive Patients.

Clinical Outcome	Cefotaxime	Ceftazidime	Gentamicin	Ciprofloxacin	Resistance pattern
Died	I	R	S	S	BSC
Unknown	I	R	R	R	MRE
Survived	S	R	N/A	S	BSC
Died	S	R	S	S	BSC
Died	I	R	N/A	S	BSC
Died	S	R	N/A	S	BSC
Survived	R	R	S	S	BSC
Died	I	R	S	R	BSC
Survived	S	S	N/A	S	Not BSC
Survived	S	R	S	S	BSC
Survived	I	R	S	S	BSC
Died	R	R	N/A	S	BSC
Died	S	R	S	R	BSC
Died	S	S	S	S	Not BSC
Died	S	S	S	S	Not BSC
Unknown	I	R	R	S	BSC
Died	N/A	R	N/A	I	BSC
Survived	S	S	N/A	S	Not BSC
Survived	R	R	S	S	BSC
Died	R	R	R	R	MRE
Survived	R	R	R	I	MRE
Died	R	R	R	I	MRE
Survived	R	R	R	I	MRE
Died	R	R	R	I	MRE
Died	N/A	R	S	R	BSC

(N/A – Antibiotic was not tested, **Unknown** – Patient was brought home against medical advice, **BSC** – Broad-spectrum Cephalosporins, **MRE** – Multiresistant Enterobacter, **S** – Sensitive, **R** – Resistant, **I** – Intermediate, **BSC resistant** – Resistant to Cefotaxime or Ceftazidime, **MRE** – Resistant to Cefotaxime or Ceftazidime, Ciprofloxacin and Gentamicin)

DISCUSSION

No microbiologic test is more important for the clinician than the blood culture in dealing with sepsis. Although only 5 to 15 percent of

blood cultures drawn in symptomatic patients are positive,[16] and out of this, only 72% are true positive results,[17] the finding of pathogenic microorganisms in the bloodstream often provides critical clinical information that in turn leads to specific, often, life-saving therapy. In this study, the positivity rate of CDUH-NICU blood cultures is 8.4 % (110/1312), which is within the expected range. However, such rate is much lower in contrast to the studies done by Mokuolu et. al., Kumhar et. al., and Rahman et. al. with positivity rates of 30.8%, 42% and 62.8%, respectively.[18,19,20] The 8.4% rate, however, may not be a true reflection of the positive blood culture result but it provides the physician practicing in said institution a baseline percentage on neonatal bacteremia.

In Nepal, second to Klebsiella spp, said organism is the most common isolate (10 out of 103 blood culture positive or 9.7%), during the period of January 2006 to February 2007 at Neonatal Division of BP Koirala Institute of Health Sciences, Dharan, Nepal (the largest tertiary care pediatric hospital in the Eastern region of Nepal).[8] It is also a growing problem in Pakistan, based on a five-year prospective surveillance of neonatal sepsis at the Aga Khan University in Karachi Pakistan, wherein 10% of neonatal sepsis was caused by Enterobacter spp. with associated mortality of 21%.[9] On the other hand, at the Neonatal Division of Dongguk University Hospital in Kyongju Korea, neonatal sepsis caused by Enterobacter spp. was 33.9% and associated mortality was 26.5 %.[10]

In a twenty-two-year study (1977 to 1998) on the increase of Enterobacter spp. in neonatal sepsis at the Departments of Neonatology and Microbiology in Unidad de Investigacion, Hospital Universitario Son Dureta, Palma de Mallorca, Spain, 8.7% of all neonatal bacteremias are due to Enterobacter spp. and 6.6% of them died. An outbreak of Enterobacter spp. was noted in the years 1992 to 1998.[11]

During the last four years, results in this study showed a significant increase in the incidence of Enterobacter spp. as a cause of bacteremia; this observation had also been stated by other authors.[8-11] Although, ICC's routine surveillance failed to identify the source of infection, the source of outbreak and the causes of the emergence of Enterobacter spp., it should also be noted that in other neonatology units the same were not identified; this is supported by the previously mentioned institution studies. Improved hygiene practices led to the progressive disappearance of the epidemic strain in those hospitals. On the other hand, a study done by Levine, et. al. (1999), showed that because extensive intrapartum chemoprophylaxis can drastically reduce Group B Streptococcus (GBS) neonatal sepsis, a concomitant increase in the incidence of gram negative neonatal sepsis was observed.[21] This may be a possibility in the researchers' hospital but data on the use of intrapartum antibiotic prophylaxis given to the mothers of the neonates included in the study was not known. It is, however, interesting to note that most of the blood culture isolates within the study period were gram negative organisms.

The researchers also found that there was a relatively high rate of resistance to broad-spectrum Cephalosporins (BSC) at 60% which is comparable to the recent published rates of 30.5% up to 93.1%.[15] The recommendation of not treating Enterobacter infections with broad spectrum Cephalosporins[15] is also suggested by the researcher since BSC resistance in this study was quite high.

One of the findings in this study was that Enterobacter spp. was most sensitive to the four antibiotics tested—Imipenem, Meropenem, Cefepime and Piperacillin-Tazobactam. The researchers, therefore, propose that in cases of documented Enterobacter bacteremia, the previously mentioned antibiotics should be the antibiotics of choice.

Even though all patients received the same empirical treatment—a combination of Ampicillin and Amikacin, later on shifted to

either, Ceftazidime and Piperacillin-Tazobactam, and lastly to Meropenem or Imipenem based on the sensitivity result of the blood culture, the clinical outcome of neonates with Enterobacter spp. and the mortality were statistically significant. Although significant, this result may not be generalizable to all patients with Enterobacter spp. bacteremia because data on the patient's factors (underlying diseases, age of gestation, birth weight) that were not included in this study may have influenced the higher mortality rate.

In evaluating the relationship between the clinical outcome and sensitivity patterns of Enterobacter spp. to MRE specifically, the result was not statistically significant at P<0.53.Although a growing number of resistant Enterobacter strains, particularly to BSC and MRE, have been observed,[15] the impact of antibiotic sensitivity and resistance on mortality is still under debate.[22-25] A study done by Marcos stated that bacteremias-associated mortality depends not only on antimicrobial therapy but also on other aspects of treatment (removal of infection foci) and patients factors, as mentioned above.[26] This may explain why the relationship of the clinical outcome of neonates with MRE is not statistically significant.

CONCLUSIONS AND RECOMMENDATIONS

The neonatal bacteremia of Enterobacter spp. in CDUH–NICU from January 2005 to December 2008 have an overall mortality rate of 56% (p<0.013), which was statistically significant; thus, continued surveillance should be maintained to control their emergence or spread. On the other hand, isolates were most sensitive to Imipenem (100%), Meropenem (92%), Cefepime and Piperacillin-Tazobactam (80%), which leads the researchers to suggest that any of these antibiotics may be used when dealing with the mentioned pathogen.

Most Enterobacter spp. isolates were BSC-resistant while 24% were MRE-resistant. However, relationship of the clinical outcome and multiresistant Enterobacter spp. was statistically not significant, probably because the outcome depends not only on antimicrobial therapy but also on other aspects of treatment and other variables such as microorganisms that may need to be studied.

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