



Prevalence of MDR pathogens in blood and bodyfluids of Paediatric patients in a tertiary care hospital.

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ABSTRACT

INTRODUCTION

Nowadays, antimicrobial resistance is a challenge faced by physicians globally. Infections due to Multidrug Resistant Organisms (MDROs) is a significant problem in Paediatric Intensive Care Unit (PICU).

AIM

To identify the burden of antimicrobial resistance in hospital isolates from paediatric patients and elucidate the resistance pattern of MDROs.

MATERIAL AND METHODS

All the clinical specimen received from pediatric outpatient and inpatient were included in the study and data were collected and processed on blood agar, chocolate agar and MacConkey agar. Cled agar from those yielding growth on urine culture. The bacterial isolates were identified with standard biochemical tests. Antibiotic susceptibility testing was done according to CLSI guidelines 2019 by Kirby Bauer disk diffusion method.

RESULTS

A total of 100 (39.07%) bacteria were isolated from 256 clinical samples, 65 bisolates were from urine followed by blood (22), exudates (9) and respiratory (4) samples. 22 were MDROs, 14 were from urine, blood (5), exudates (2) and respiratory (1) samples respectively. Isolates of *E. coli* (54%), *Klebsiella* spp (36%), and *Enterococcus* (0.9%) spp. were the common MDROs. The most sensitive antibiotic for gram negative bacteria was Polymyxin-B (100%) followed by Imipenem (98%) and Meropenem (90%). The most sensitive antibiotic for gram positive bacteria was Linezolid (100%), Vancomycin (97%) followed by Teicoplanin (94%).

CONCLUSION

This study reveals significant prevalence of MDR pathogens among paediatric population. *E. coli*, *Klebsiella* spp. and *Enterococcus* spp. were the common MDROs encountered in the study, mostly isolated from urine culture.

KEY WORDS :- Bacterial, Drug resistance, *cons* , septicemia .

INTRODUCTION

Antimicrobial resistance is on the rise, creeping into the paediatric population too. Infections due to MDROs is a significant problem in paediatric ICU. Multidrug-resistant organisms (MDROs), including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and certain gram-negative bacilli (GNB) have important infection control implications that either have not been addressed or received only limited consideration in previous isolation guidelines. Increasing experience with these organisms is improving understanding of the routes of transmission and effective preventive measures. Although transmission of MDROs is most frequently documented in acute care facilities, all healthcare settings are affected by the emergence and transmission of antimicrobial-resistant microbes. *Staphylococcus aureus* is a major cause of bacterial infections in humans worldwide. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a versatile pathogen capable of causing a wide variety of human diseases. The epidemiology of MRSA is constantly changing, which results in variation in its drug-resistance patterns throughout regions and countries^{1,2,3} Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of bacteremia and invasive diseases that include pulmonary, skin, and soft tissues and musculoskeletal infections in children.^{4,5}

Material and Method

Material

256 Samples of paediatric patients were collected., from patients admitted to the ICU and NICU of Rama Medical college, hospital and research centre The study was conducted from January 2019 to December 2019.

Method

All the samples were collected from pediatric outpatient and inpatient were included and collected in MacConkey agar, Cled agar from those yielding growth on culture. The bacterial isolate was identified with standard bio- chemical test.

Preliminary identification was done with the help of the following methods:- Gram Stain:- The suspected colonies were stained using gram stain method and their shape, colour, and arrangement were observed under light microscope.

Procedure of Gram Staining: -Gram Staining is a differential staining and it consists of four steps: Primary stain (Crystal violet) for 1 minute. Mordant (Gram's iodine) for 1 minute. Decolourizer is – (acetone) for 5 sec. Counter stain (safranin) for 30s.

INCLUSION CRITERIA:- Paediatric patients with clinical infection and sample, Pus, urine, blood, body fluid were collected. Social demographic data, medical history, clinical history regarding age, gender type of paediatric patient percentage and antibiotic therapy was taken.

EXCLUSION CRITERIA:- Repeat isolates from the same patients and from the same site/specimen all were excluded.

BIOCHEMICAL TESTS CATALASE TEST:

Principle - This test demonstrates the presence of catalase an enzymes that catalyses the release of oxygen from hydrogen peroxide.

Procedure- Take a clean grease free slide. Divide it into two halves. One half serves as test and the other as control. Put one drop of 3% H₂O₂ each on the test and control halves Now, with the help of wooden stick a separate colony from the culture plate and touch on the surface of H₂O₂ marked as test. Look for the effervescence production. P.C-ATCC *Staphylococcus aureus* (25923) N.C-ATCC *Enterococcus faecalis* (29212)

STATISTICAL ANALYSIS:-

Statistical analysis was performed by using computer-based software, Statistical Package for Social Science (SPSS). Mean values of parameters were compared to determine the result.

Observation & Result

In out of 256 samples total 100 sample were found with various bacterial growth which mean prevalence of bacteria was 39.88% in our study. The prevalances of different type of bacteria n our study was *CONS* (5.46%), *Stapylococcus aureus* (7.03%). *E.coli* (18.35%), *Klebsiella spp* (7.42%) *Pseudomonas aeruginosa* (5.46%), *Enterococcus spp* (14.84%).

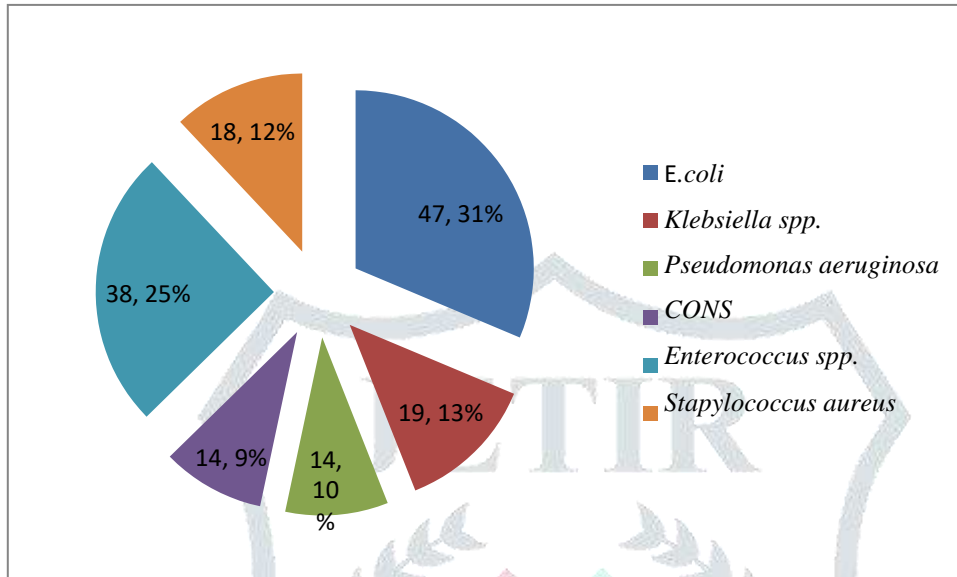


Fig No1 Number of different bacteria found in total sample

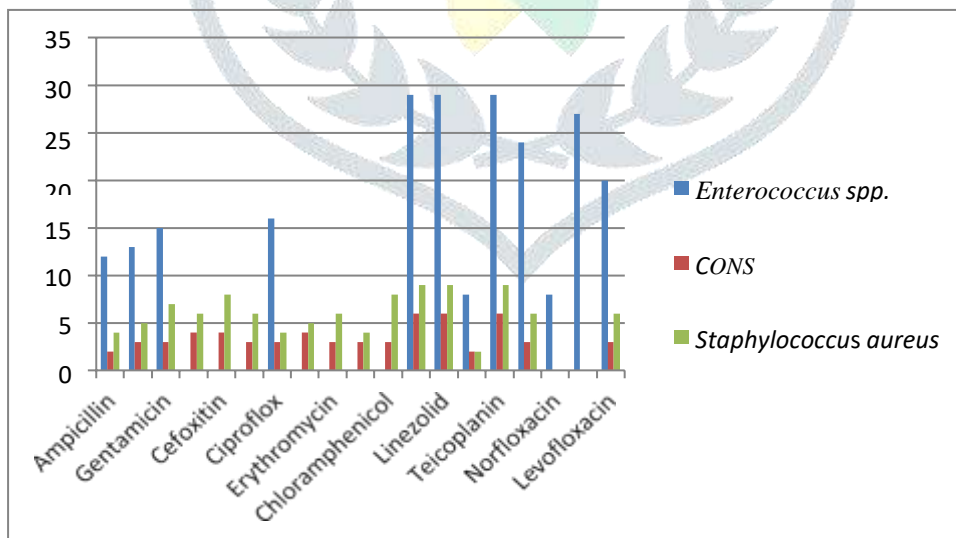


Fig 2 showing the antibiotic sensitivity for gram positive cocci

In case of *CONS*, Vancomycin ,Linezolid ,Teicoplanin showed (100%) Sensitivity followed by, Tetracycline (75%) ,Cefoxitin (80%) &Levofloxacin(75%). In case of *Staphylococcus aureus*,Vancomycin Linezolid ,Teicoplanin showed (100%) sensitivity followed by, Tetracycline (40 %), Cefoxitin (50%) &Levofloxacin(40%), Gentamicin(60%). In case of *Enterococcus spp.*, Vancomycin Linezolid, Teicoplanin showed 100% sensitivity followed by, Tetracycline (75%) , Nitrofurantoin (75%).

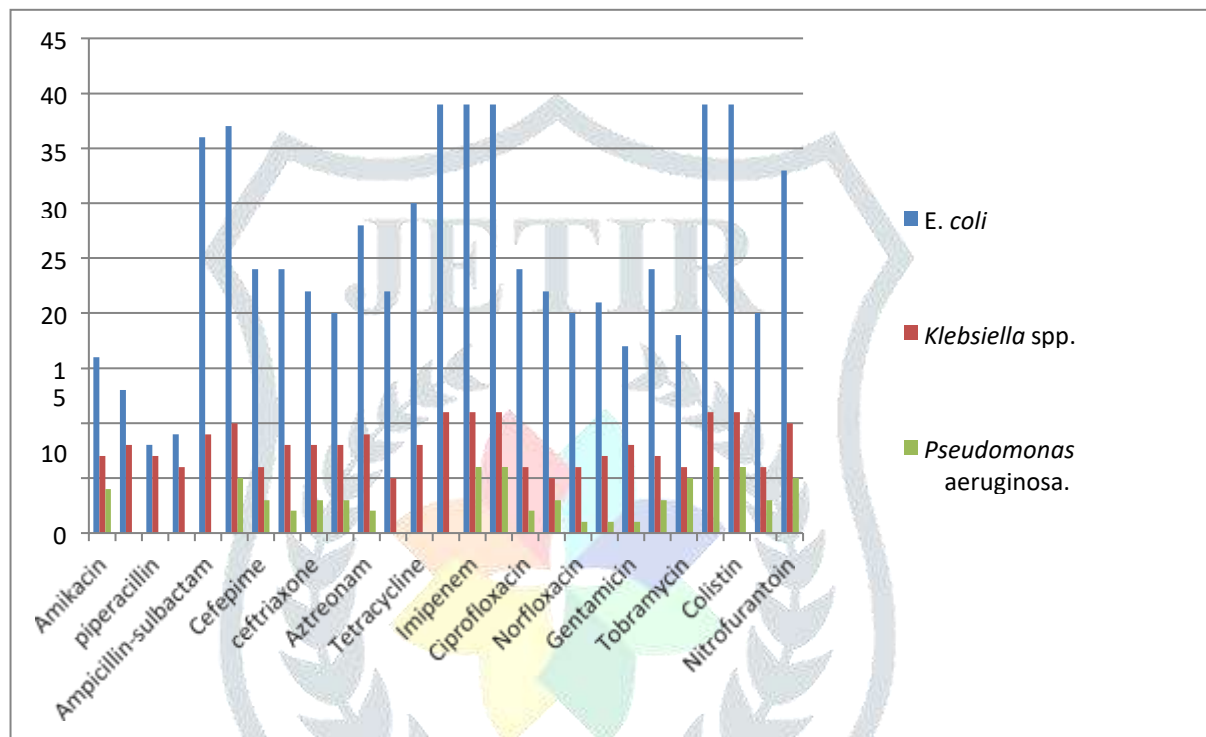


fig No 3 showing antibiotic sensitivity for gram negative cocci

In case of *E.coli*, Polymyxin-B ,colistin, Imipenem, Meropenem, Tigecycline , showed 100% Sensitivity followed by Amplicillinsulbactam(83%) ,Piperacillintazobactam(93%), Nitrofurantion(93.25%). In case of *Klebsiella spp.*,Polymyxin-B, colistin, Imipenem, Meropenem, Tigecycline, showed 100% Sensitivity followed by Ampicillinsulbactam(83%) ,Piperacillintazobactam (93%), Nitrofurantion(93.25%), Netilmycin (75%). In case of *Pseudomonas aeruginosa*, Polymyxin-B ,colistin, Imipenem, Meropenem, showed 100% Sensitivity followed b, Piperacillintazobactam(93%), Nitrofurantion(93.25%).

Discussion

In the study done by Khanal et al¹⁰ incidence of *Streptococcus* & *Staphylococcus* reported was 25 % and gram negative bacilli 15 % . But in most of the studies gram negative organisms have taken over the gram positive organisms especially in the hospital settings . In our study *S.aureus* was isolated in 27.37% of cases & *CONS* in 20.16.% of the cases. However, Roy et al¹² have reported *CONS* 16.5% and *S.aureus* 14% in neonatal septicemia.

The antimicrobial resistance profile of both GP and GN isolates showed a high prevalence of resistance among them. *Cons* and *Enterococcus spp* isolates showed higher level of resistance to beta-lactam antibiotics than *Staphylococcus aureus*. However all the three were sensitive to Vancomycin which is similar to other studies^{12,21} .

In the study done by Durgesh et al⁷, *Staphylococcus aureus* was the predominant (31.25%) isolates causing UTI, followed by *Escherichia coli* (25%), *Pseudomonas aeruginosa* (15.62%), *Proteus mirabilis* (15.62%), *Klebsiellapneumoniae* (6.25%) and *Serratiamarcescens* (6.25%) where as in our study the most common organism causing UTI was *Escherichia coli* (15.23%) isolates followed by *Enterococcus spp.* (11.23%), *Klebsiella species* (4.29), *Staphylococcus aureus* (3.51%), *Pseudomonas aeruginosa* (2.34%), *CONS* (2.34), Similarly, in the study done by Yolbas et al⁶ and Mostafa et al⁸ , *Escherichia coli* was the most common organism causing urinary tract infection followed by *Klebsiella* which was similar to our study. Similar results were observed in studies done by Rehaman et al⁹ and CW kwan et al¹⁰. This difference may be due to the variation in geographical distribution.

A Study done by Haller et al showed the effective empirical intravenous and oral antibiotics for the treatment of community-acquired UTIs include Ampicillin and Aminoglycosides¹¹, whereas the oral antibiotic of choice by Prais D et al¹² and Hoberman et al¹³ showed Amoxicillin-Clavulanate or Cefuroxime and Cefixime respectively.

Compared to the study done by Yolbas et al⁶, in which *Staphylococcus aureus* was resistant to Amikacin in 3%, and Cefotaxime 51%, in our study *Escherichia coli* showed more resistant pattern to these antibiotics Amikacin (15.6%), Nitrofurantoin (46.8%), Cotrimoxazole (68.75%) and Cefotaxim (81%). The reason for increase in resistance may be due to the excessive use of third generation Cephalosporins both as oral and intravenous route.

In the study done by Durgesh et al⁷, the mean sensitivity to Penicillin and Ciprofloxacin were 70.83% and 60% respectively. *Staphylococcus aureus* showed 95 % resistance to Methicillin, Oxacillin and Vancomycin. blood pathogens were sensitive to Norfloxacin, Co- trimoxazole and Ofloxacin. These results were in contrast to our study in which *Escherichia coli* was sensitive to Amoxicillin and Ciprofloxacin in only 6% and 15.6% respectively, and in case of *Klebsiella pneumoniae* none was sensitive to Amoxicillin

and only 40% were sensitive to Ciprofloxacin. The reason for this difference may be due to the injudicious over the counter use of antibiotics.

In the study done by Mostafa et al⁸, *CONS* had a sensitivity rate of 97.8% to vancomycin and 95.2% to Linezolid in contrast to our study in which vancomycin was sensitive only in 18.7% of cases. The highest resistance rate of *CONS* was to Penicillin (95.2%) followed by Amoxycillin and Cotrimoxazole (79 and 74.2% respectively) in the study by Mostafa et al¹¹ whereas in our study *CONS* showed high resistant to Amoxycillin (93%) followed by Nalidixic acid (87.5%). According to Mostafa et al⁸ *Klebsiella* spp. showed the highest sensitivity to Ciprofloxacin (95.1) and Ceftriaxone 90.7% which was in contrast to our study in which *Klebsiella* was sensitive to Ciprofloxacin and Cefotaxim only in 40% and 30% respectively. In our study *Klebsiella* showed highest resistant to Amoxycillin which was similar to study done by Mostafa et al⁸.

In Study done by Reham et al⁹, 59.9% isolates of *Escherichia coli* were multidrug resistant whereas in our study only one *CONS* was multidrug resistant. In our study *Escherichia coli* showed increase in resistance to oral antibiotics; Amoxycillin (93%), Ofloxacin (81.2%), Ciprofloxacin (84.3%) Cotrimoxazole (68.75%), and Nitrofurantoin (46.8%) which was similar to the results in study done by Reham et al⁹. The reason may be easy access to oral antibiotics from pharmacy.

In our study *CONS* sensitive to Cefotaxim in only 18.75% whereas in study done by CW Kwan et al¹⁰, *Escherichia coli* was sensitive to greater than 95% of third-generation Cephalosporins (Ceftriaxone and Ceftazidime). In their study bacteria were frequently resistant to ampicillin (54.4%) and Trimethoprim-Sulfamethoxazole (40.4%) which was comparable to our study.

In our study *Escherichia coli* (81.2%) and *Klebsiella pneumoniae* (70%) showed increase in resistance pattern to Cephalosporins which was comparable to study done by Stephanie A et al¹⁴ in which there was high rate of resistance to third generation Cephalosporins in subpopulations of children admitted to the hospital for UTIs.

In the study done by Rasoulet al¹⁵, most isolates showed high resistance against Ampicillin, Cotrimoxazole, Nalidixic acid, and Nitrofurantoin and *Klebsiella* isolates showed more resistance against tested antibiotics than *Escherichia coli* isolates which was comparable to our study

Although our study suggested high resistance to oral antibiotics, there was a caveat that this study included in-patients only. This might have caused some selection bias. However, this information raises an important issue regarding antibiotic resistance in Septicemia. A future study including both in-patients and out-patients would help clarify if resistance to oral antibiotics has indeed emerged in the organisms causing septicemia in the community.

Conclusion

This study reveals significant prevalence of MDR pathogens among paediatric population ESBL (*E.coli*), MBL (*P.aeruginosa*), MRSA (*Staphylococcus aureus*) & VRE (*Enterococcus spp.*). *E. coli*, *Klebsiella spp.* and *Enterococcus spp.* were the common MDROs encountered in the study, most of the isolates were from urine culture. MDROs are present among outpatients as well, warranting judicious use of antibiotics and adequate infection control measures to prevent spread of these potential pathogens. Knowledge of the antibiotics susceptibility pattern of the isolates from paediatric patient infection is crucial for planning the appropriate treatment of these cases prior to getting the susceptibility reports from the laboratory

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