RESEARCH PAPER

Carbapenem-resistant *Enterobacteriaceae* in Pediatric Bloodstream Infections in Rural Southern India

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Objective: To measure the frequency of antimicrobial resistance in pediatric blood culture isolates of *Escherichia coli* and *Klebsiella* spp. with focus on carbapenem resistance. **Methods:** Over a period of three years, pediatric blood culture isolates were tested for antimicrobial susceptibility, including molecular investigations for carbapenem resistance. **Results:** Amikacin, carbapenems, colistin and tigecycline had an antimicrobial efficacy of >70% (n=140). 7 of the 15 randomly selected isolates were positive for *carbapenemase* gene; among them, five were New Delhi Metallo β-lactamase (NDM). **Conclusion:** There was a high prevalence of *Klebsiella* spp. in pediatric bacteremia and dissemination of NDM-mediated carbapenem resistance in pediatric wards.

Keywords: *Antimicrobial resistance, Blood culture, Escherichia coli, Klebsiella spp.*

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Bloodstream infections (BSI) are important causes of morbidity and mortality in children [1]. Successful outcome of these BSI depends on prompt and timely administration of appropriate antimicrobials. Drug-resistant organisms, especially the carbapenemase-producing strains of *Escherichia coli* and *Klebsiella* spp. are concerning pediatric health care providers recently [2-4].

Common causes of pediatric BSI and their antimicrobial resistance (AMR) patterns are highly variable across institutions [5]. Therefore, the surveillance of pathogens causing pediatric BSI and understanding their local epidemiology of AMR is an important prerequisite to predict empirical therapy. This study aimed to measure the prevalence of pathogens causing BSI in pediatric population of rural Southern India, and to measure the frequency of AMR in *E.coli* and *Klebsiella* spp. isolates.

METHODS

This cross-sectional study was conducted at a tertiary care teaching hospital from January 2012 to December 2014. Blood culture specimens received for bacteriological investigation from pediatric (age 0 to 12 years) inpatients as part of their routine patient management were included in this study. The study protocol was approved by the Institutional Review Board of Government Theni Medical College.

Blood culture and the identification of isolate was performed as per the standard procedure [6]. The culture was considered contaminated when any of the following organisms was identified: *Micrococcus* spp., viridans streptococci, *Bacillus* spp., and diphtheroids [7].

Antimicrobial susceptibility test was performed by Kirby-Bauer disk diffusion method (zone of inhibition) by following Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. For colistin (≥11 mm) and tigecycline (>18 mm), alternative susceptibility breakpoints were used, as these were not available from CLSI [9,10].

During the study period, randomly selected ertapenem (ETP) non-susceptible isolates were tested for Minimum Inhibitory Concentration (MIC) of carbapenems (ETP, imipenem, meropenem, and doripenem by agar dilution method) [8], and for the carriage of carbapenemase encoding genes (CEG) such as *bla*NDM (New Delhi Metallo β-lactamase), *bla*KPC (*Klebsiella pneumoniae* Carbapenemase), *bla*OXA-48 (Oxacillinase), *bla*VIM (Verona Integron-encoded Metallo β-lactamase) and *bla*IMP (Imipenemase) by multiplex PCR using previously published primers [11].

Isolates not susceptible to any of the carbapenems other than ETP by MIC was defined as Carbapenem-resistant *Enterobacteriaceae* (CRE) [12].

Frequency of AMR was calculated by WHONET software and statistical analysis was performed with Chi-square test and the difference was considered significant when P value was less than 0.05.

RESULTS

A total of 1932 pediatric patients were tested by blood culture during the study period. Among them, 880 (45.5%) were positive with true pathogen (***Table* I**), of which 38 and 102 isolates were identified as *E. coli* and *Klebsiella* spp., respectively. Contaminants were identified in 316 (16.4%) of blood culture specimens.

The cumulative antimicrobial susceptibility data revealed high proportion of resistance in both *E. coli* and *Klebsiella* spp. to the most of the antibiotics tested (***Table* II**). Amikacin, carbapenems, colistin and tigecycline had an antimicrobial efficacy of >70%. When compared with *E. coli*, the resistance rate in *Klebsiella* spp. was higher to most of the antibiotics; specifically, significant difference was observed with piperacillin, cefazolin, cefuroxime, ceftazidime, aztreonam, gentamicin, and colistin. However, the resistance rate to fluoroquinolones, doxycycline, cefoxitin, cefepime, and piperacillin/tazobactam were lower in *Klebsiella* spp. than *E. coli* among which the significant difference was only observed with cefoxitin.

TABLE I CULTURE ISOLATES FROM PEDIATRIC BLOODSTREAM INFECTIONS (*N*=880)

Organism	*No. of isolates (%)*
Gram Positive Bacteria	
Staphylococcus aureus	77 (8.8)
Staphylococcus , Coagulase Negative	333 (37.8)
Streptococcus spp.	10 (1.1)
Enterococcus spp.	67 (7.6)
Gram Negative Bacteria	
Escherichia coli	38 (4.3)
Klebsiella pneumoniae	98 (11.1)
Klebsiella oxytoca	4 (0.5)
Proteus mirabilis	10 (1.1)
Proteus vulgaris	4 (0.5)
Enterobacter spp.	9 (1.0)
Citrobacter spp.	18 (2.1)
Morganella spp.	2 (0.2)
Providencia spp.	3 (0.3)
Hafnia spp.	5 (0.6)
Serratia spp.	2 (0.2)
Salmonella spp.	15 (1.7)
Shigella spp.	2 (0.2)
Pseudomonas aeruginosa	21 (2.4)
Non-fermenting Gram-negative bacilli	121 (13.8)
Gram-negative cocci/coccobacilli	12 (1.4)
Fungi	
Candida spp.	29 (3.3)

Thirty-one isolates (24 *K. pneumoniae* and 7 *E. coli)* were not susceptible to ETP; among them, randomly selected 15 isolates (13 *K. pneumoniae* and 2 *E. coli*) were further tested. Nine were CRE and seven were CEG positive (5 of them had NDM, one of which also had KPC; further, one each were KPC and VIM; intriguingly, three of these isolates are not CRE). Further, among the nine CRE isolates, only four were CEG positive (3 NDM, and 1 NDM + KPC), and remaining five isolates may be resistant by other mechanisms not tested in this study. Interestingly, among the six non-CRE isolates, three were found positive for CEG (one each for NDM, KPC, and VIM).

Among these 15 cases, nine were successfully treated (2 were NDM, 1 was NDM + KPC and 1 was VIM positive; 5 were CEG negative), and three patients died (two were preterm with low birth weight, NDM positive; and third was full term with asphyxia/respiratory failure, CEG negative); and in remaining three, outcome could not be assessed. None of these 15 isolates was pan-drug resistant; notably, 13/15 (86.7%) isolates were susceptible to colistin and all of them were susceptible to tigecycline.

DISCUSSION

We documented significantly higher proportion of Klebsiella when compared with *E. coli* as the cause of BSI. The most effective antibiotic (*E. coli* and *Klebsiella* spp. combined) in the study was colistin (92.8%), followed by tigecycline (89.3%). Carbapenems (>77%) were sufficiently effective for the consideration of empirical therapy for Gram-negative bacterial sepsis [13]. Further, this report documents the emergence of CRE in pediatric wards in rural Southern India; also, substantiates that the presence of CEG need not confer clinical resistance to carbapenem. Thus, the testing of MIC is more important than the detection of CEG in terms of patient management [14].

Limitation of the study was that it was done at single center, which may not reflect the overall picture in India; however, it may be useful to forecast the prevalence of

TABLE II *IN VITRO* ACTIVITY OF ANTIMICROBIAL AGENTS AGAINST PEDIATRIC BLOOD CULTURE ISOLATES OF *E. COLI* AND *KLEBSIELLA* SPP.

Antimicrobial name	*E. coli (n=38) %R (95% CI)*	*Klebsiella spp. (n=102) %R (95% CI)*	*P value**
Ampicillin	90.6 (73.8-97.5)	94.9 (88.0-98.1)	0.75
Piperacillin	60 (36.4-80.0)	89.6 (79.1-95.4)	<0.01
Amoxicillin/Clavulanic acid	73.9 (51.3-88.9)	84.9 (75.2-91.4)	0.07
Piperacillin/Tazobactam	31.2 (16.7-50.1)	27.5 (18.9-38.0)	0.47
Cefazolin	82.1 (62.4-93.2)	96.6 (89.6-99.1)	<0.01
Cefuroxime	61.5 (40.7-79.1)	88.2 (78.9-93.9)	<0.01
Ceftazidime	69.7 (51.1-83.8)	82 (72.8-88.7)	<0.01
Cefotaxime	71.4 (51.1-86.0)	92.4 (84.5-96.6)	0.05
Cefepime	51.7 (32.9-70.1)	38.6 (28.6-49.6)	0.16
Cefoxitin	46.2 (20.4-73.9)	21.2 (11.6-35.1)	<0.01
Aztreonam	59.4 (40.8-75.8)	71 (60.5-79.7)	0.04
Doripenem	10 (0.5-45.9)	14.3 (6.0-29.2)	0.34
Ertapenem	13.3 (4.3-31.6)	22.4 (14.8-32.2)	0.14
Imipenem	11.1 (2.9-30.3)	11.5 (6.0-20.6)	0.76
Meropenem	10 (0.5-45.9)	16.3 (7.3-31.3)	0.18
Amikacin	21.2 (9.6-39.4)	24 (16.3-33.8)	0.61
Gentamicin	30 (15.4-49.6)	66 (55.4-75.3)	<0.01
Nalidixic acid	48.4 (30.6-66.6)	59.8 (48.7-70.0)	0.31
Ciprofloxacin	42.4 (25.9-60.6)	39.8 (30.2-50.2)	0.66
Gemifloxacin	43.3 (25.9-62.3)	38.2 (28.3-49.1)	0.58
Levofloxacin	30 (15.4-49.6)	20 (12.6-30.0)	0.2
Ofloxacin	32.3 (17.4-51.5)	27 (18.8-37.0)	0.16
Trimethoprim/Sulfamethoxazole	71 (51.8-85.1)	78.1 (68.3-85.6)	0.23
Colistin	0 (0-28.3)	8.6 (3.2-19.7)	<0.01
Doxycycline	36 (18.7-57.4)	19.3 (12.0-29.4)	0.12
Tetracycline	56.7 (37.7-74.1)	65.6 (55.0-74.9)	0.5
Tigecycline	0 (0.0-25.3)	1.6 (0.1-9.9)	0.15

**For difference in proportion of resistance.*

resistance in rural or similar resource-limited settings. Additionally, most patients were referred for blood culture only after the failure of empiric therapy, which might have prejudiced the high resistance rate observed in this study.

In conclusion, the present study documented the higher prevalence of *Klebsiella* spp. in pediatric BSI and emergence of CRE; this necessitates the strengthening of infection control measures and effective antibiotic policy to contain their spread in pediatric wards.

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Contributions: RS and S Srivani: Concept, study design, data analysis and manuscript preparation; MM: Designed the study, and provided critical inputs to manuscript; DS and SL: Acquisition of data, analysis and interpretation of data; S Saranya: Laboratory testing, data collection and documentation**.** *Funding*: Partly supported by an ad-hoc research grant offered by Indian Council of Medical Research (grant number 5/3/3/21/2012-ECD-1); *Competing interests*: None stated.

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WHAT THIS STUDY ADDS?

- There is emergence of NDM-producing Carbapenem-resistance *Enterobacteriaceae* in pediatric bloodstream infections.

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