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ORIGINAL RESEARCH

Antimicrobial Resistance and Resistance Determinant Insights into Multi-Drug Resistant Gram-Negative Bacteria Isolates from Paediatric Patients in China

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Introduction: The emergence of multi-drug-resistant Gram-negative bacteria (GNB) is a concern in China and globally. This study investigated antimicrobial resistance traits and resistance determinant detection in GNB isolates from paediatric patients in China.

Methods: In the present study, a total of 170 isolates of GNB including the most prevalent *Escherichia coli, Klebsiella pneumoniae* and *Acinetobacter baumannii* were collected from Shenzhen Children's Hospital, China. ESBLs production was confirmed by using the combination disc diffusion method, and carbapenemase production was confirmed by using a carbapenem inactivation method followed by antimicrobial susceptibility. In addition, β-lactamase-encoding genes and co-existence of plasmid-borne colistin resistance *mcr-1* gene were determined by PCR and sequencing.

Results: Overall, 170 etiological agents (GNB) were recovered from 158 paediatric patients. The most prevalent species was *E. coli* 40% (n=68), followed by *K. pneumoniae* 17.64% (n=30), and *Enterobacter cloacae* 14.11% (n=24). Of 170 GNB, 71.76% (n=122) were multi-drug-resistant, 12.35% (n=21) extreme-drug resistant, and 7.64% (n=13) single-drug-resistant, while 8.23% (n=14) were sensitive to all of the studied antibiotics. The prevalence of ESBLs and carbapenemase producers were 60% and 17%, respectively. $bla_{\text{CTX-M}}$ was the most prevalent resistance gene (59.42%), followed by bla_{TEM} (41.17%), bla_{SHV} (34.270%), bla_{KPC} (34.11%), $bla_{\text{OXA-48}}$ (18.82%) and $bla_{\text{NDM-1}}$ (17.64%).

Conclusion: The present study provides insights into the linkage between the resistance patterns of GNB to commonly used antibiotics and their uses in China. The findings are useful for understanding the genetics of resistance traits and difficulty in tackling of GNB in paediatric patients.

Keywords: Gram-negative bacteria, antimicrobial susceptibility, ESBLs, carbapenemase, molecular characterization

Introduction

The emergence of infectious diseases caused by multi-drug-resistant (MDR) pathogens is a major problem in the community, especially in children.[1,2] MDR or extreme drug-resistant (XDR) GNB contributes to global infectious diseases in paediatric patients.[3] Recent reports have shown that the rate of resistance in GNB increases periodically worldwide.[4] The genomic adaptions: acquisition of resistance determinant by horizontal gene transfer and/or spontaneous mutation in the genome

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are two major mechanisms that confer resistance against antibiotics in bacteria.[5,6] The genomic mutation is responsible for modification in target sequences, overexpression of target, ie, efflux pump and reduced intake of antibiotics, while acquired resistance traits can modify the target post-translationally, inactivate antibiotics by hydrolysis or chemical modification, or may provide alternative metabolic pathways, etc.[7] MDR or XDR GNB is more notable in developing countries due to the restricted antibiotics, indiscriminate use of the drugs, poor hygiene, dietary deficiency and poor governing supervision.[8,9] However, the antimicrobial resistance problem is still underestimated because of inadequate or ineffective diagnosis in some clinical settings.[9,10] Extended spectrum β-lactamases (ESBLs) and carbapenemases are key resistance. These are a group of plasmids-borne, heterogeneous, complex and rapidly evolving enzymes which are capable of hydrolysing penicillin, cephalosporin, aztreonam and monobactams.[11,12] According to Bush-Jacoby-Medeiros classification, ESBLs have been classified into three major groups: TEM, SHV and CTX-M, while carbapenemases enzymes encoded by alleles of the bla_{KPC} gene depict one of the five substantial carbapenemase families, others being the VIM, IMP and Delhi Metallo-β-lactamase (MβL) (NDM), and the OXA-48-like oxacillinases.[13–15] The β-lactamase production is most commonly seen among GNB including *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*) *and Pseudomonas aeruginosa* (*P. aeruginosa*).[16] In the past few years, high dissemination of ESBLs and carbapenemases-producing GNB were observed worldwide and alarm to developing countries. Children with MDR bacteraemia are more likely to receive inadequate initial antibiotic therapy and have a higher rate of infectious complications and death.[17] These observations suggest a critical need for the promotion of antimicrobial stewardship and reduction in unnecessary antibiotic use and avoid the horizontal gene transfer in the paediatric patients.

Methods

Bacterial Isolation and Identification

A total of 170 non-duplicate clinical isolates (GNB) were collected from 158 patients between October 2018 and May 2019 from Shenzhen Children's Hospital (SCH), China. This hospital is a major children hospital in the southern area of China. A single specimen was isolated from n=146 (85.88%) paediatric patient's samples, while two specimens were isolated from n=12 (7%) patient's samples. Among the 170 GNB, 54.12% (n=92) were from male and 45.88% (n=78) were from female; patients' age ranges from ≥4 months to 12 years. The criteria used for inclusion of the isolates in the present study are as follows: first, isolates must be the Gram-negative. Second, the pathogens may link with the community or hospital-associated infections. Bacterial isolates belonging to family Enterobacteriaceae including *E. coli, K. pneumoniae, Enterobacter cloacae* (*E. cloacae*), *Proteus vulgaris* (*P. vulgaris*) were isolated on MacConkey Agar (Becton Dickinson, USA), *Salmonella species* were cultivated on deoxycholate citrate agar (Merck, USA), *A. baumannii* cultivated on CHROMA™ *Acinetobacter* agar (Merck, USA), while *Elizabethkingia meningoseptica* (*E. meningoseptica*), *Burkholderia cepacia* (*B. cepacia*) cultivated on blood agar, and *P. aeruginosa* were cultivated on cetrimide agar (Merck, USA). Sets of biochemical tests were performed to identify isolates. The precise phylogenetic identity of all the GNB isolates was further confirmed by 16S rRNA gene sequencing. Bacterial species used in this study were isolated and characterized in biological safety cabinet Class II Type. The origin of the specimens was as follows: urine n=58, sputum n=51, pus n=38, blood n=18, catheter-associated (CA) n=2, and cerebral spinal fluid (CSF) n=3 (S-1).

Phenotypic Detection of ESBLs Production

The combination disc test was done for phenotypic detection of ESBLs production. The test was performed by using a disc of both cefotaxime and ceftazidime, alone and in combination with clavulanic acid. Control strain was selected from the characterized strain collection of our laboratory, while ATCC25922 was used as a negative control strain. The ESBLs production result was analysed according to the Clinical and Laboratory Standards Institute (CLSI) guideline.[18]

Phenotypic Detection of CRKP

Carbapenemase production was confirmed by using a newly developed Carbapenem Inactivation Method (CIM) which was first delineated in the year 2015.[19] To carry out CIM, an antibiotic susceptibility-testing disc of 10-μg meropenem (MEM) was incubated for 2 hrs in an aqueous suspension of a *K. pneumoniae*. The disc was removed from the suspension and placed onto a Mueller-

Hinton agar (MHA) plate seeded with an ATCC25922 indicator organism; followed by overnight incubation, the zone of inhibition was measured to determine whether the MEM has been hydrolysed (growth of the indicator organism under 14 mm area), or still active (>14 mm large zone of inhibition around the disk). Control strain was selected from the characterized strain collection of our laboratory.

Antimicrobial Susceptibility Patterns

Antimicrobial susceptibility was performed by VITEK@2 compact system (Biomerieux-Ref. No.27530/275660) method for 21 antimicrobial agents, namely, amoxicillin, amikacin, aztreonam, ceftazidime, ciprofloxacin, ceftriaxone, colistin, cefuroxime, cefuroxime axetile, cefazolin, ertapenem, cefepime, cefoxitin, imipenem, levofloxacin, nitrofurantoin, ampicillin/sulbactam, trimethoprim/sulfamethoxazole, tigecycline, tobramycin, piperacillin-tazobactam. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline[18] and colistin susceptibility was determined according to EUCAST.

Detection of Antimicrobial Resistance Encoding Genes

The standard PCR assay was performed to detect the presence of resistance encoding genes: ESBLs encoding genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{GES}, bla_{CARB}, bla_{PER}, bla_{VEB} and bla_{OXA}) using specific primers previously described (Table 1). In addition, carbapenemase genes (bla_{KPC} and bla_{NDM}) and colistin resistance *mcr-1* genes were determined by PCR assay and sequencing. The specific primers were used as described in our previous study (Table 1).[20–22] The purified PCR products were sequenced commercially (Sangon Biotech-Shanghai, China). DNA Sequences were analysed by NCBI-BLAST program.

Data Analysis and Statistical Tests

Data were double-entered to Epi Data version 3.1 and transferred to SPSS version 20 and Microsoft Excel software for analysis, and the results were presented as tables, pie-charts and graphs. P-values < 0.05 were considered as statistically significant.

Results

Bacterial Confirmation

A total of 170 GNB most commonly *E. coli* 40% (n=68), *K. pneumoniae* 17.64% (n=30), *E. cloacae* 14.11% (n=24), *P. aeruginosa* 12.94% (n=22), *A. baumannii* 5.88% (n=10) (Figure 1).

Resistance Trends for Commonly used Antibiotics

We summarized the overall resistance trends of commonly used antibiotics against all the isolated GNB over the study period (Figure 2A and B). Overall, ceftriaxone resistance was observed to be the highest 54.11% (n=92). In addition, It has resistance to trimethoprim/sulfamethoxazole 49.41% (n=84), cefuroxime 48.23% (n=82), ampicillin sulbactam 41.76% (n=71), cefazolin 34.70% (n=59), aztreonam 33.52% (n=57), ciprofloxacin 28.82% (n=49), cefoxitin 22.94% (n=39), amoxicillin 19.41% (n=33), ceftazidime 17.64% (n=30), levofloxacin 15.88% (n=27), nitrofurantoin 14% (n=25), cefepime 12.94% (n=22), tobramycin 10.58% (n=18), tigecycline 9.41% (n=16), piperacillin-tazobactam 8.23% (n=14), imipenem and ertapenem 5.29% (n=9) each, amikacin 2.35% (n=4) colistin 1.76% (n=3) (Figure 2A and B). From isolated strains, *E. coli* has having resistance against ceftriaxone about 54% (n=37), followed by *K. pneumoniae* 50% (n=15) and *E. cloacae* 50% (n=12), *Salmonella spp*. 50% (n=4), *P. aeruginosa* 45% (n=10), while *C. freundii* were found sensitive to cephalosporin group of antibiotics. Other side *S. typhimurium* showed highest resistance for cephalosporin about 63% (n=5) followed by *E. cloacae, K. pneumoniae, E. coli* about 58% (n=14), 53% (n=16), and 49% (n=33) respectively, Table 2. Conversely, *E. coli*, K. *pneumoniae*, and *P. aeruginosa* isolates were very low resistance for aminoglycoside group (Table 2).

Resistance Patterns for the Different Isolates

Our results reflected that resistance diversity of the pathogens was not observed in any distinct trends. Among 170 GNB, 71.64% (n=122) shown that MDR phenotype followed by 12.35% (n=21) isolates was shown XDR phonotype, 7.64% (n=13) SDR and 8.23% (n=14) MDS phenotype. Form a total of 68 *E. coli* isolates, 6 (9%), 8 (12%), 41 (60%) and 13 (19%) were multi-drug sensitive (MDS), single-drug resistance (SDR), MDR, XDR, respectively. The overall prevalence of MDR among all isolates was E. *cloacae* 96% (n=23) followed by K. *pneumoniae* 80% (n=24), *P. aeruginosa* 77% (n=17) and *A. baumannii* 60% (n=6) Table 3. The

Table 1 List of Primers Used in This Study

Resistance Genes	Primers Pair Sequences	Amplicon Size (bp)	Annealing Temperature (°C)	References
mcr-1	ATGATGCAGCATACTTCTGTG TCAGCGGATGAATGCGGTG	1626	56	20
bla_{NDM}	TGCGGGGTTTTTAATGCTG TGGCTCATCACGATCATGC	785	53	21
bla_{KPC}	ATGTCACTGTATCGCCGTC TTACTGCCCGTTAACGCC	883	54	21
bla_{TEM}	AGGAAGAGTATGATTCAACA CTCGTCGTTTGGTATGGC	531	57	21
bla_{SHV}	GGTTATGCGTTATATTCGCC TTAGCTTTGCCAGTGCTC	866	57	21
bla_{OXA48}	TTGGTGGCATCGATTATCGG GAGCACTTCTTTTGTGATGGC	745	55	21
bla_{SME}	AACGGCTTCATTTTTGTTTAG GCTTCCGCAATAGTTTTATCA	831	55	21
bla_{CMY}	CTGACAGCCTCTTTCTCCA GCCAAACAGACCAATGCT	504	56	21
bla_{VIM}	GTTAAAAGTTATTAGTAGTTTATTG CTACTCGGCGACTGAGC	799	60	21
bla_{IMP}	ATGAGCAAGTTATCTGTATTC TTAGTTGCTTGGTTTTGATGG	741	60	21
bla_{GES}	ATGCGCTTCATTCACGCAC CTATTTGTCCGTGCTCAGG	864	57	22
bla_{CARB}	AAAGCAGATCTTGTGACCTATTC TCAGCGCGACTGTGATGTATAAAC	588	56	22
bla_{PER}	AGTCAGCGGCTTAGATA CGTATGAAAAGGACAATC	978	56	22
bla_{VEB}	GCGGTAATTTAACCAGA GCCTATGAGCCAGTGTT	961	57	22
bla_{CTX-M}	TTTGCGATGTGCAGTACCAGTAA CGATATCGTTGGTGGTGCCATA	544	57	22

prevalence of XDR among all the isolates were *A. baumannii* 20% (n=2), K. *pneumoniae* 10% (n=3), *P. aeruginosa* 9% (n=2). The based on the combination disc test result, 61.76% (n=105) ESBLs producers were identified and carbapenem inactivation method test result confirms that 17% (n=30) were carbapenemase producer. A significant association was observed between ESBLs producers and carbapenemase producer (*P*-value is 0.003642). No significant association was observed between resistance classes (MDS, SDR, MDR, XDR) and isolated GNB isolates (*P* value is 0.83077).

Genomics of Resistant Isolates

We explored genome to characterise resistance genes from the GNB. Screening resistance genes showed that Gram-negative isolates harboured $bla_{\text{CTX-M}}$ 59.41% (n=101) with most common being $bla_{\text{CTX-M-15}}$ (n=44), $bla_{\text{CTX-M-65}}$ (n=20), $bla_{\text{CTX-M-90}}$ (n-12), $bla_{\text{CTX-M-14}}$ (n=11), $bla_{\text{CTX-M-3}}$ (n=6), $bla_{\text{CTX-M-27}}$ (n=4), $bla_{\text{CTX-M-98}}$, $bla_{\text{CTX-M-211}}$ and $bla_{\text{CTX-M-64}}$ (n=1each) (S-2). Additionally, other β-lactamase encoding genes were detected, bla_{TEM} 41.11% (n=70), bla_{SHV} 34.70% (n=59), bla_{KPC} 34.11% (n=58), $bla_{\text{OXA-48}}$ 18.82% (n=32), $bla_{\text{NDM-1}}$ 17.64% (n=30),

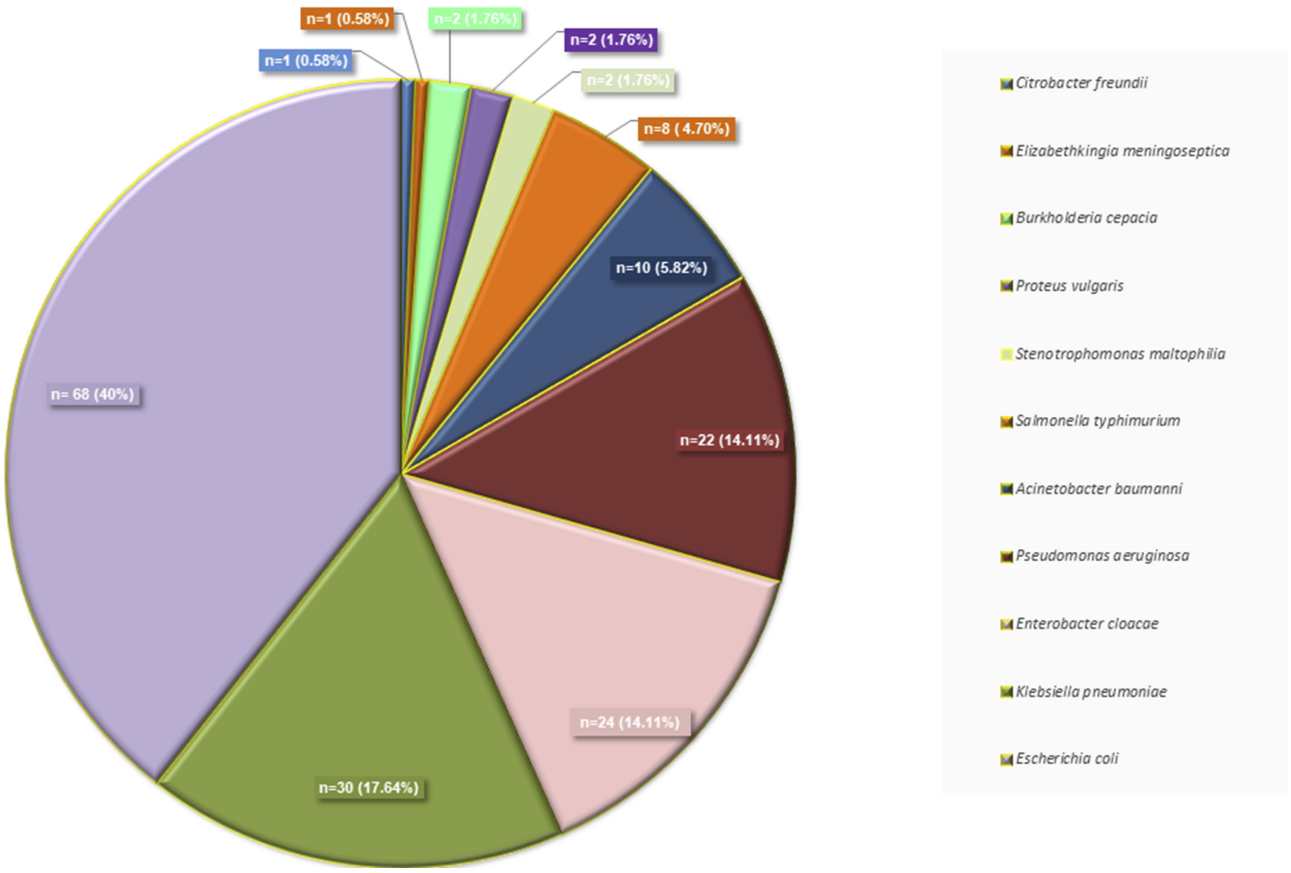

Figure 1 Distribution of isolated Gram-negative pathogens from Shenzhen Children's Hospital, China.

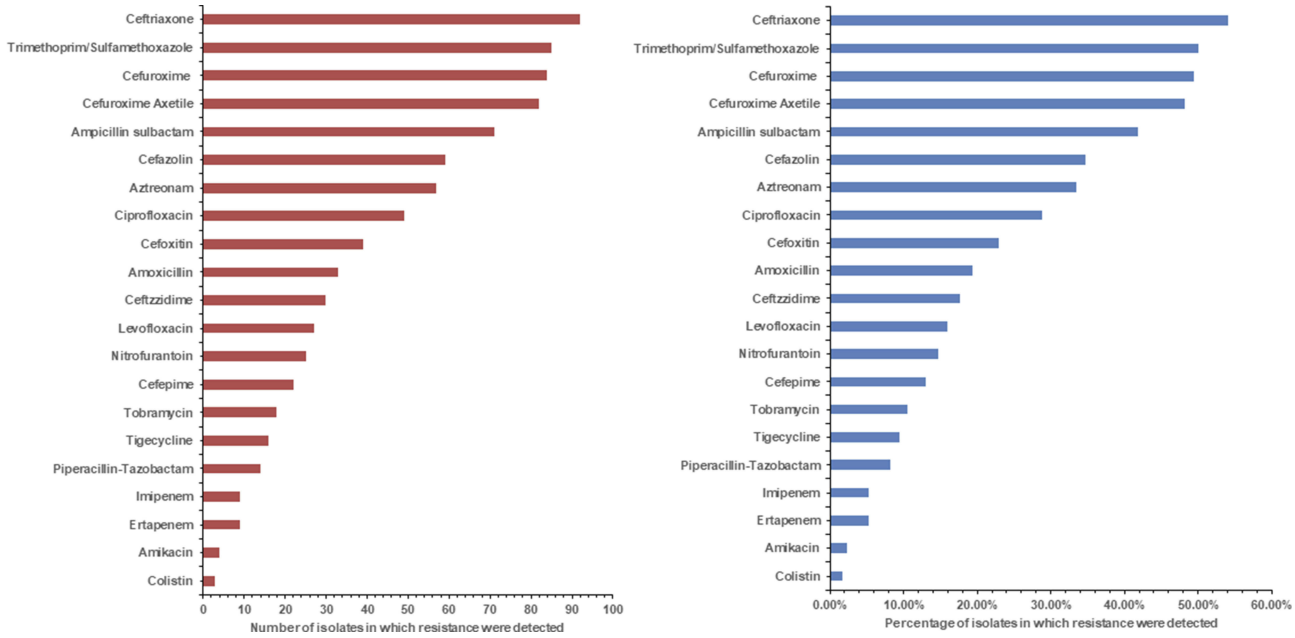

Figure 2 (**A**) Number of isolates in which resistance is detected for each antibiotic. (**B**) Detection percentages of resistance to each antibiotic.

bla$_{GES}$ 14.11% (n=24), *bla*$_{VIM}$ 9.41% (n=16), *bla*$_{CARB}$ 8.23% (n=14) most recently discovered plasmid-borne colistin resistance *mcr-1* 1.76% (n=3) Table 4. The *bla*$_{PER}$, *bla*$_{VEB}$ and *bla*$_{SME}$ genes were not detected. The significant

Table 2 Resistance Patterns of Gram-Negative Bacteria for Selected Antibiotics and Antibiotics Classes

Antibiotic Classes	Name of Antibiotics	*E. coli* (n=68)	*K. pneumoniae* (n=30)	*E. cloacae* (n=24)	*P. aeruginosa* (n=22)	*A. baumannii* (n=10)	*S. typhimurium* (n=8)	*S. maltophilia* (n=2)	*P. vulgaris* (n=2)	*B. cepacian* (n=2)	*E. meningoseptica* (n=1)	*C. freundii* (n=1)
Cephalosporin	Ceftazidime	15	1	4	4	1	0	1	0	0	1	0
	Ceftriaxone	37	15	12	10	2	4	1	1	2	0	0
	Cefuroxime	33	16	14	8	1	5	1	0	2	1	0
	Cefuroxime Axetile	38	13	13	9	2	3	1	2	2	1	0
	Ertapenem	5	3	0	2	0	0	0	0	0	0	0
	Cefepime	14	4	2	1	0	1	0	0	0	0	0
	Cefoxitin	17	10	3	4	0	1	0	0	0	0	0
	Cefazolin	24	15	5	8	1	4	0	2	1	0	0
Penicillin	Amoxicillin	18	7	3	4	0	0	0	0	1	0	0
	Ampicillin sulbactam	30	11	6	13	5	3	0	1	0	1	1
	Piperacillin-Tazobactam	8	3	2	2	1	0	0	0	0	0	0
Aminoglycoside	Amikacin	1	2	1	0	0	0	1	0	0	0	0
	Tobramycin	8	6	1	2	1	0	0	0	0	0	0
	Imipenem	3	2	0	1	0	0	0	0	0	0	0
Quinolones	Ciprofloxacin	25	10	8	7	2	1	0	0	1	1	0
	Levofloxacin	16	4	1	5	2	1	0	1	1	0	0
Monobactam	Aztreonam	16	10	10	8	2	1	0	1	1	0	0
Nitrofuran Derivative	Nitrofurantoin	13	5	3	3	2	2	0	0	0	1	1
Sulphonamides	Trimethoprim/ Sulfamethoxazole	39	12	9	12	7	1	0	1	0	1	1
Polymyxin	Colistin	1	1	0	1	0	0	0	0	0	0	0
Tetracycline	Tigecycline	4	6	1	1	1	1	1	0	0	0	0

Table 3 Distribution of MDS, SDR, MDR, and XDR Pattern of Gram-Negative Bacteria

Isolated Strains	Total	MDS	SDR	MDR	XDR
C. freundii	1	0	1	0	0
E. meningoseptica	1	0	0	1	0
B. cepacia	2	0	0	2	0
P. vulgaris	2	0	0	2	0
S. maltophilia	2	0	0	2	0
S. typhimurium	8	2	1	4	1
A. baumannii	10	2	0	6	2
P. aeruginosa	22	1	2	17	2
E. cloacae	24	0	1	23	0
K. pneumoniae	30	3	0	24	3
E. coli	68	6	8	41	13
Total	170	14	13	122	21

Notes: MDS, susceptible to all antibiotic classes; SDR, resistant to single antibiotic class; MDR, resistant to at least one agent in three or more antimicrobial categories; XDR, resistant to at least one agent in all but two or fewer antimicrobial categories (ie, bacterial isolates remain susceptible to only one or two categories). Source: Based on definitions by Magiorakos et al, 2012.[23]

co-associations were observed between bla$_{CTX\text{-}M}$ to bla$_{TNDM\text{-}1}$, bla$_{KPC}$, and bla$_{TEM}$ (p = 0.003462, 0.00001 and 0.000335, respectively).

Discussion

Infection caused by GNB is one of the major burdens on low and middle-income countries because of acquisition of resistance genotype. GNB, more precisely, *E. coli, K. pneumoniae, E. cloacae, P. vulgari, Salmonella species*, account for the most severe forms of infections including urinary tract infection (UTI), bacteraemia and pneumonia. The usual therapeutic approaches to treat such conditions are by antibiotics.[24,25] Recent findings on antimicrobial resistance revealed that resistances to frontline antimicrobials among GNB are very common and the resistance can spread from multiple sources through a number of pathogenic or commensal microbes by horizontal gene transfer.[26] The overall rate of MDR and XDR of the GNB from SCH were found to be 71% and 12.0%, respectively. Furthermore, the observed MDR rate is significantly associated with prolonged hospital stay and the patients, who die due to MDR bacterial species (even if, it is not statistically significant). On the other hand, the observed XDR rate at our hospital indicates that the problem of antimicrobial resistance is increasing at an alarming rate and circulating Gram-negative pathogenic bacteria in SCH are becoming more resistant to available all available antibiotics. Recent reports from Wenzhou, China have indicated that 60.1% MDR gram-negative pathogen infection in the children's

Table 4 Prevalence of Drug Resistance Determinant (Genes) in Isolated Gram-Negative Bacteria

Drug Resistance Determinant	*E. coli* (n=68)	*K. pneumoniae* (n=30)	*E. cloacae* (n=24)	*P. aeruginosa* (n=22)	*A. baumanni* (n=10)	*S. typhimurium* (n=8)	*S. maltophilia* (n=2)	*P. vulgaris* (n=2)	*B. cepacian* (n=2)	*E. meningoseptica* (n=1)	*C. freundii* (n=1)	Total n=170
mcr-1	2	0	0	0	0	1	0	0	0	0	0	3
bla$_{CTX\text{-}M}$	37	19	18	14	6	3	2	0	1	1	0	101
bla$_{NDM\text{-}1}$	15	6	5	1	2	0	0	0	0	0	1	30
bla$_{KPC}$	21	12	12	8	01	1	1	0	1	0	1	58
bla$_{TEM}$	30	16	9	7	3	3	0	0	1	1	0	70
bla$_{SHV}$	16	16	12	7	3	3	0	0	1	1	0	59
bla$_{GES}$	11	3	1	5	2	2	0	0	0	0	0	24
bla$_{CARB}$	5	2	2	2	0	1	1	0	1	0	0	14
bla$_{PER}$	0	0	0	0	0	0	0	0	0	0	0	0
bla$_{VEB}$	0	0	0	0	0	0	0	0	0	0	0	0
bla$_{OXA48}$	14	4	7	0	1	2	1	1	1	1	0	32
bla$_{SME}$	0	0	0	0	0	0	0	0	0	0	0	0
bla$_{VIM}$	4	6	2	2	1	1	0	0	0	0	0	16

hospital is almost similar to our results.[27] The occurrence of a high rate of MDR gram-negative pathogenic bacteria would also have huge potential threat and implications for children's care in the hospital and the community at large. As we are living in the Shenzhen (International City) of a very connected world, it is highly likely for these MDR bacteria to be disseminated to other parts of China and globally.

To the best of our knowledge, there is no previous report from Shenzhen on the rate of MDR and XDR gram-negative pathogenic bacteria from paediatric patients and genetic resistance determinant analysis to compare with these results. The antimicrobial susceptibility test data clearly indicate that a high resistance rate of the gram-negative pathogen to the cephalosporin group of antibiotics has raised a serious concern and become a challenge for clinicians. Therefore, we suggest avoiding indiscriminate use of antibiotics in medical practice which will certainly lower the opportunities for the emergence of resistance. Our antimicrobial susceptibility results are comparable to another part of China, Taiwan, and the USA.[28,29] The ESBLs detection test confirms 59% (n=101) ESBLs producers, while 17% were carbapenemase producer which is quite higher than our previous study,[17] but comparable to studies done in Nigeria, Nepal and India.[30,31] Development of AMR is an outcome of complex microbial interactions and resistance may arise by the acquisition of de-novo mutation during clinical antibiotic treatment or commonly by the acquisition of integrative or replicative mobile genetic elements (MGEs) that have evolved over time in microbes in the natural ecosystem. The environmental reservoirs of resistance genes are widely diverse and similar resistance genes may present in distantly related bacterial species, which can co-exist in the same habitat.[32,33] It was reported that *K. pneumoniae, V. cholerae, E. coli, P. aeruginosa*, and *Salmonella* were naturally competent and can uptake naked DNA from the environment in suitable conditions.[34,35] In this study, we observed that $bla_{\text{CTX-M}}$ as the most prevalent genotype of ESBLs-producing gram-negative pathogens in SCH, which is composed of $bla_{\text{CTX-M-15}}$ followed by $bla_{\text{CTX-M-65}}$, $bla_{\text{CTX-M-90}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-98}}$, $bla_{\text{CTX-M-211}}$ and $bla_{\text{CTX-M-64}}$ (S-2). This result indicates the diversity of CTX-M genotype of ESBLs-producing gram-negative pathogens spread in Shenzhen, China. Additional, bla_{TEM} followed by bla_{SHV}, $bla_{\text{OXA-48}}$, bla_{GES}, bla_{CARB} and bla_{VIM} genes exists in isolated GNB. Similar results were reported from Japan and Tanzania.[36,37] In our study, $\text{bla}_{\text{CTX-M-55}}$ is not detected normally in pediatric patients, which means children may not be in contact with an animal since this genotype is mostly circulated via animal origin *E. coli* isolates.[38] We do not observe bla_{VEB}, bla_{PER} and bla_{SME} from same isolates. We observed that bla_{KPC} 34% and $bla_{\text{NDM-1}}$ 17.64% as the most prevalent carbapenemases encoding genes in GNB. In addition, co-existence of ESBLs encoding $bla_{\text{CTX-M}}$ genes with carbapenemases encoding genes bla_{KPC} or $bla_{\text{NDM-1}}$ raises a concern about the spread of superbugs in the Shenzhen. Several reports mentioned the co-existence of $bla_{\text{CTX-M}}$ with bla_{KPC} or $bla_{\text{NDM-1}}$ globally.[39] We observed three isolates harbouring plasmid-borne colistin resistance *mcr-1*, even highly spreading over tChina.[40] The overall study suggested that limited options are available to treat MDR GNB infection in children.

Conclusion

In past few years, a number of studies have been repeated globally on antimicrobial resistance focused on pathogenicity, prevalence, resistance mechanisms (acquisition and dissemination of resistance genes), as well as on drug-susceptibility testing, rapid diagnosis of AMR pathogens and developing a strategy to re-sensitize the resistance variants against existing drugs of public-health importance. Despite the availability of the plethora of information on the fundamental science of resistance biology, the information on the rise of resistant pathogens across the globe is surprisingly scarce. Our data show that the clinical isolates (gram-negative pathogen) are continuously evolving to counterbalance the bactericidal/bacteriostatic effects of clinically important antimicrobial drugs because of acquisition of resistance elements such as $bla_{\text{CTX-M}}$, bla_{KPC}, $bla_{\text{NDM-1}}$, bla_{TEM}, bla_{SHV}, $bla_{\text{OXA-48}}$, bla_{GES}, bla_{VIM} and bla_{CARB}. It is a right time to develop strategies for robust surveillance, restriction on improper antibiotic usage and identify the routes that are facilitating the rapid dissemination of antibiotic resistance in pathogenic and non-pathogenic bacterial cells in children's hospitals.

Ethics Statement

The present study was approved by the ethical committee of the Shenzhen Children's Hospital (Research) 2018 (013). The clinical isolates used in this research were part of routine hospital laboratory procedures. Verbal consent was given by the patient's parent/s or legal guardian/s.

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Disclosure

The authors report no conflicts of interest in this work.

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