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Original article

Ceftazidime/avibactam efficiency tested *in vitro* against carbapenem-resistant *Klebsiella pneumoniae* isolated from neonates with sepsis

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ABSTRACT

Background: Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) emergence and dissemination, is an important healthcare concern due to its limited therapeutic options. Ceftazidime/avibactam (CAZ/AVI) is a recently approved antibiotic combination that may be effective in treating these resistant infections**.** The aim of the current study was to determine the prevalence of CRKP among clinical isolates from neonatal intensive care unit (NICU) and to evaluate the *in-vitro* activity of CAZ/AVI against them. **Methods:** A total of 255 clinical samples were collected from neonates with suspected sepsis. All *Klebsiella pneumoniae* isolates were identified by standard methods. Antibiotic susceptibility testing, screening for Extended-spectrum β lactamase (ESBL) production, carbapenem resistance, carbapenemase production and susceptibility to CAZ/AVI were done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results**: Of the 255 neonates clinically suspected as neonatal sepsis, only 136 (53.3%) had positive culture results, out the 136 culture-proven cases, 72 (52.9%) were positive for *Klebsiella pneumoniae*, of them ESBL producers were 92% (n=66) and CRKP were 32% of isolates (n=23). All of the CRKP were carbapenamase producers (39% serine-type and 61% metallo-β-lactamases (MBL) type). Serine carbapenamase and MBL producers showed high resistance against CAZ/AVI with a percentage of 77.8% and 100% respectively. **Conclusion**: The prevalence of CRKP is alarming in our NICU especially in the presesnce of neonatal risk factors like; neutropropenia, central line fixation and premature rapture of membranes. Ceftazidime/avibactam is an unsuitable option for the treatment of this type of resistant bacteria because of its high resistance.

Introduction

Klebsiella pneumoniae (K. pneumoniae) is a Gram-negative, rod-shaped, lactose fermenter bacterium that belongs to the *Enterobacteriaceae* family that causes many types of nosocomial infections [1]. Many risk factors increase the *K. pneumoniae* infections in Neonatal Intensive Care Units (NICUs) including low birth weight (LBW), premature rupture of membranes (PROM), prematurity, neutropenia, intravascular catheterization, nutrition by the parenteral route, intubation, tracheostomy and prolonged hospitalization [2].

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Nour et al. have stated that the mortality rate owing to Gram-negative bacterial infections in (NICUs) ranges somewhere in the range of 10% and 67.7% and worsens in the presence of bacteremia, resistance to carbapenem, inappropriate/postponed empirical antimicrobial therapy, and underlying illness [3]. Carbapenems are effective antibacterial agents that are used in severe infections caused by multi-drug resistant *Enterobacteriaceae* e.g. *K. pneumoniae* as carbapenems are often considered final-resort antibiotics for severe *K. pneumoniae* infections [2].

The recent development and rapid spread of carbapenem-resistant *K. pneumoniae* (CRKP) are therefore of global concern [4]. *Klebsiella pneumoniae* carbapenemase is the main resistance mechanism in *K. pneumoniae*. Carbapenamases are hydrolyzing beta-lactamases encoded by transmissible plasmids, which help and facilitate its spread among the different bacterial species. Carbapenemases are broadly divided into two major types based on the amino acid sequences: Metallo β-lactamases (MBLs) containing zinc at the active site (Ambler class B) and serine β-lactamases containing serine at the active site (Ambler classes A, C, and D)[5].

The rough mortality rate due to CRKP infections ranges from 30% to 44%. It exacerbates strikingly to 71.9%, in bacteremia cases [6]. Few drug options, like polymyxins, tigecycline, and fosfomycin, may be effective against CRKP. However, these antibiotics are rarely used as a monotherapy to treat CRKP infections, either because of its complex pharmacokinetics or toxicity or unknown optimal therapeutic doses or resistance or finally due to high mortality rate [7-10].

The mortality rate associated with CRKP infection and the restricted antimicrobial alternatives for the treatment of them highlight the requirement for improved, rapid, and simple detection of CRKP infection and the advancement of novel agents with reliable clinical efficacy against them [11,12]. The modified carbapenem inactivation method (mCIM) is an effective and simple phenotypic test, it became the Clinical and Laboratory Standards Institute (CLSI) recommended method in 2017. Its results are highly reliable with the presence of carbapenemase genes detected with polymerase chain reaction (PCR) (100% concurrent for *Enterobacteriaceae*) [13].

In 2017, The World Health Organization defined carbapenem-resistant *Enterobacteriaceae* (CRE) as one of the highest priority pathogens for the development of new antibiotics [14]. Ceftazidime/avibactam (CAZ/AVI) is a recently approved antibiotic combination that may be efficient in treating these resistant infections. Avibactam is a novel β-lactamase inhibitor (BLI) combined with ceftazidime, a cephalosporin with an established history. Avibactam can inhibit a wide range of β-lactamases, including Ambler Class A (GEM, SHV, CTX-M, and KPC), Class C (AmpC), and some Class D (OXA- 48) β-lactamases. (CAZ–AVI) has been utilized in the United States since 2015 and CLSI recommended its use in 2018 [11, 15].

In Egypt, carbapenem resistance is evolving and threatens [16,17], hence this study was carried out to determine the prevalence of CRKP among *K. pneumoniae* neonatal isolates and to investigate the *in-vitro* activity of new CAZ-AVI against these isolates.

Material and Methods

Study location and design

This cross-sectional, prospective, single-center study was performed in the NICU and the Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Egypt. This study was conducted from September 2019 to May 2020, and has been approved by the Institutional Review Board (IRB) and Ethical Committee of Faculty of Medicine, Zagazig University. It was conducted according to the revised declaration of Helsinki. Consents were obtained from patients' parents upon sample collection.

Patients, samples collection, and processing

Patients included in this study were neonates aged from birth to 1 month who were incubated in NICU and had *K. pneumoniae* growth in their blood or endotracheal aspirate or urine cultures. Any patient with congenital anomalies incompatible with survival was excluded from the study. Patients information were collected from their files including; demographic data (gestational age and sex), birth weight, delivery method, presence of PROM, perinatal fever, neutropenia, central venous catheter (CVC) insertion, intubation and mechanical ventilation (MV).

Supposing that prevalence rate of neonatal sepsis with positive culture is 50% [18] and attendance rate of neonates with sepsis was 423 patients per six months so the sample was calculated to be 232

patients. Ten % of calculated sample was added to compensate for potential drop out; hence a total of 255 patients were included. Sample size was calculated using open Epi program with power of study 80% and confidence level 95%. A total of 255 clinical samples were collected from patients including blood for blood culture, endotracheal aspirate (ETA), and urine. Briefly, 1–2 ml of blood was collected for culture into 8 ml blood culture bottles; Egyptian diagnostic media ® for isolation of bacteria from the blood. ETA samples were collected by direct endotracheal suction of respiratory secretions using sterile suction catheters. Subcultures of the blood culture bottles and samples of endotracheal aspirates were inoculated on (sheep blood agar, chocolate agar, and MacConkey media). Urine samples were collected by sterile urine bags and inoculated as early as possible on a surface of CLED medium [3].

Identification of isolated bacteria and anti-microbial susceptibility testing

Identification of bacterial isolates was done by conventional methods which included, colonial morphology, Gram-stained films, and biochemical reactions including indole, oxidase, motility, citrate, and urease tests. Anti-microbial susceptibility testing was performed using the modified Kirby-Bauer disc diffusion method according to the standards of the CLSI [19]. The antibiotic discs (Bioanalyse®) used for susceptibility testing were: penicillin derivatives with β-lactamase inhibitor combinations; Amoxicillin/ clavulanic acid (AMC) 20/10ug, ampicillin/sulbactam (SAM) 10/10ug, and piperacillin/tazobactam 100/10ug (TZP). Cephalosporins: Cefuroxime (CXM) 30ug, ceftriaxone (CRO) 30ug, cefotaxime (CTX) 30ug, Ceftazidime (CAZ) 30ug, cefoperazone (CPE) 75ug and cefepime (FEP) 30ug, Monobactams; aztreonam (ATM) 30ug. Carbapenems: Meropenem (MEM) 10ug and imipenem 10ug (IPM), Aminoglycoside: Amikacin 30ug (AK) and gentamicin 10ug (CN), fluoroquinolone: Ciprofloxacin (CIP) 5ug and levofloxacin (LEV) 5ug. *Escherichia coli* ATCC® 25922 and *K. pneumoniae* ATCC® 700603 were used as standard control strains.

We confirmed the identification and antimicrobial susceptibility of a few randomly selected *K. pneumoniae* strains in the Clinical Pathology Department, Faculty of Medicine, Zagazig University, by VITEK 2 automated systems (bioMerieux, France).

Phenotypic determination of antibiotic resistant *K. pneumoniae* strains

Multi-drug resistant (MDR) strains were defined as acquired non-susceptibility to at least one drug in three or more antimicrobial classes, extremely drug-resistant (XDR) was defined as non-susceptibility to all antimicrobial classes except one or two categories, and Pan-drug resistant (PDR) was defined as non-susceptibility to all drugs in all antimicrobial classes [20]. Detection of MDR, XDR, and PDR strains was performed depending on the results of the disc diffusion method.

Phenotypic determination of ESBL producing isolates

Extended-spectrum β lactamase (ESBL) producing isolates have been identified phenotypically by the previous standard disc diffusion procedure for the four antimicrobial discs; Ceftazidime 30 ug, cefotaxime 30ug, ceftriaxone 30ug, and aztreonam 30ug as described by the CLSI 2020 [19]. If the zones of inhibition were less than 22 mm, 27 mm, 25 mm, and 27 mm respectively the *K. pneumoniae* isolate was considered ESBL producer. We used a *K. pneumoniae* ATCC 700603® control strain as a QC strain for this procedure, its standard zones of inhibition for the four tested antimicrobials discs were 10-18 mm, 17-25 mm, 16-24 mm, and 10-16 mm respectively.

Phenotypic detection of carbapenem-resistant and carbapenamases producers' strains

Carbapenem-resistance was defined by the US CLSI as non-susceptibility to either meropenem, imipenem, or ertapenem antimicrobials [19]. So *K. pneumoniae* isolates that weren't susceptible to imipenem and/ or meropenem discs were carbapenem-resistant strains and were phenotypically screened for carbapenamases production by modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM) as outlined by CLSI 2020 [19]. By mCIM we determined the carbapenemase activity of the tested bacteria on the carbapenem disk by measuring the diameter of the inhibition zone of E. coli ATCC 25922 after disc inactivation by the tested bacterium. Both the sensitivity and specificity of mCIM were 100% [13]. The eCIM has a sensitivity of > 95% and a specificity of >92% for differentiation of metallo-β-lactamases (NDM, VIM, and IMP) from serine carbapenamases (KPC, OXA, and SME) among Enterobacterals [19].

Determination of sensitivity to ceftazidime-avibactam antibiotic

Besides the previous panel of antibiotics tested, the new agent ceftazidime/avibactam (30/20 ug) obtained from (Liofilchem, Italy) was tested against carbapenem-resistant isolates using the disc diffusion method. For Enterobacterales, the CLSI ceftazidime–avibactam breakpoints are ≥ 21 mm susceptible and ≤ 20 mm resistant.

Statistical analysis

Statistical analysis was performed using (Statistical package for Social Science) SPSS software version16.0. Terms of numbers and percentages were used for representation of categorical variables. The distribution Differences in categorical variables were calculated using the Chi square and Fisher exact test. A *p value < 0.05* was considered statistically significant and *p > 0.05* was considered non-significant.

Results

During the study period, 423 cases were admitted to the NICU; 255 (60.3%) were clinically suspected as neonatal sepsis. Of the 255 neonates, only 136 (53.3%) had positive culture results, so the percentage of confirmed neonatal sepsis concerning the total admitted cases is (32.2%). The male-to-female ratio of the babies with sepsis was 3:2. The mean birth weight was 2.3 ± 0.67 Kg (1 – 4.2) and the mean age was 2.32 ± 5.46 days (1- 24). Of the 136 culture-proven cases of neonatal sepsis, 72 (52.9%) were positive for *K.pneumoniae*, while the remaining 64 were positive for other species of bacteria as shown in **table (1)**.

The antimicrobial resistance types for the 72 isolates of *K. pneumoniae* bacteria was as follow; MDR (n=34, 47.2%), XDR (n=26, 36.1%), and susceptible *Klebsiella* (n= 12, 16.6%) strains was detected **(Table 2)**.

Klebsiella pneumoniae isolates showed the highest resistance to penicillin (ampicillin-sulbactam, 100%, amoxicillin-clavulanate, 99% and piperacillin-tazobactam, 99%), cephalosporins (cefuroxime, 97%, ceftriaxone, 92%, cefotaxime, 92%, ceftazidime, 92%, cefoperazone, 92% and cefepime, 90%), monobactams (Aztreonam 92%) high rate of resistance was evident to aminoglycoside (gentamycin, 70%, and amikacin 60%). While the least resistance was observed with quinolones (ciprofloxacin, 50%, Levofloxacin 30%,) and carbapenems (imipenem, 32%, and meropenem, 29% **) (Figure 1)**.

A high prevalence of ESBL production was detected in the *K. pneumoniae* isolates reaching 92% **(Figure 2)**.

The prevalence of CRKP was 32% of the isolated *Klebsiella pneumoniae* **(Figure 3)**.

As regard the carbapenemase production, 39% of CRKP were serine carbapenamase producers (n=9) and 61% were MβL producers (n=14), and as regard the CAZ/AVI susceptibility, 77.8% of serine producers and 100% of MβL producers were resistant to it **(Table 3)**.

As regard the risk factors associated with CRKP sepsis PROM, neutropenia, and CVC fixation were significantly associated with it than the other tested risk factors **(Table 4)**.

Table 1. Species (SPP.) of isolated bacteria from positive cultures.

Sample / Bacterial SPP.	Positive Blood culture, (n=100), (73.5%)	Positive ETA Culture, (n=21) (15.4%)	Positive Urine Culture (n=15), (11%)	Total
Klebsiella pneumoniaea	57	11	4	72 (52.9%)
Escherichia coli	4	2	4	10 (7.4%)
Pseudomonas aeruginosa	4	1	2	7 (5.1%)
Acinetobacter	6	2	2	10 (7.4%)
Staphylococcus aureus	9	0	1	10 (7.4%)
CONS	19	3	2	24 (17.6%)
Streptococcus pnemoniaea	1	2	0	3 (2.2%)
Total	100	21	15	136(100%)

CONS: Coagulase negative Staphylococci.

Table 2. Degree of resistance of *K. pneumoniae* isolates.

Resistance type / Sample type	MDR (n=34), (47.2%)	XDR (n=26), (36.1%)	(Non- MDR, Non- XDR or PDR) (n=12), (16.6%)
Blood culture	26	21	10
ETA culture	6	4	1
Urine culture	2	1	1
Total	34	26	12

Table 3. Types of carbapenamases produced by CRKN, detected by mCIM and eCIM and its susceptibility to CAZ/AVI.

CRKP (n=23)		
Carbapenamase type (No. & %)	**Susceptibility to CAZ/AVI**	
	Sensitive (No. & %)	**Resistant (No. & %)**
Serine carbapenamase (n=9, 39%)	2 (22.2 %)	7 (77.8 %)
Metallo-β lactamase (n=14, 61%)	0 (0%)	14 (100 %)

Table 4. Association of clinical risk factors with sepsis caused by CRKP.

Risk Factors		**Carbapenem resistance No. (%)**		**Test**	***p-value***
		CRKP 23 (32%)	**CSKP 49 (68%)**		
Prematurity No. 60 (83.3%)	Yes	20 (87%)	40 (81.6%)	Fisher exact	0.8 NS
	No	3 (13%)	9 (18.4%)		
PROM No. 25 (34.7%)	Yes	17 (74%)	8 (16.3%)	Chi-square 22.9	**<0.001***
	No	6 (26%)	41 (83.7%)		
Perinatal fever No. 15 (20.8%)	Yes	6 (26%)	9 (18.4%)	Chi square 0.56	0.45 NS
	No	17 (74%)	40 (81.6%)		
Neutropenia No. 5 (6.9%)	Yes	4 (17.4%)	1 (2.1%)	Fisher exact	**0.01***
	No	19 (82.6%)	48 (97.9%)		
CVC No. 35 (48.6%)	Yes	21 (91.3%)	14 (28.6%)	Fisher exact	**<0.001***
	No	2 (8.7%)	35 (71.4%)		
Parenteral nutrition No. 36 (50%)	Yes	12 (52.2%)	23 (46.9%)	Chi Square 0.17	0.67 NS
	No	11 (47.8%)	26 (53.1%)		
Intubation and MV No. 64 (88.9%)	Yes	22 (95.7%)	43 (87.8%)	Fisher exact	0.38 NS
	No	1 (4.3%)	6 (12.2%)		
Delivery No. 18 (25%)	NVD	9 (39.1%)	9 (18.4%)	Chi-square 3.59	0.057 Ns
	CS	14 (60.9%)	40 (81.6%)		

LBW: Low birth weight, PROM: premature rupture of membranes, CVC: Central vascular catheterization, MV: mechanical ventilation, NVD: Normal Vaginal Delivery, CS: cesarean section, NS means statistically non-significant,* means significant difference.

Figure 1. Resistance pattern of isolated *K. pneumoniae* bacteria.

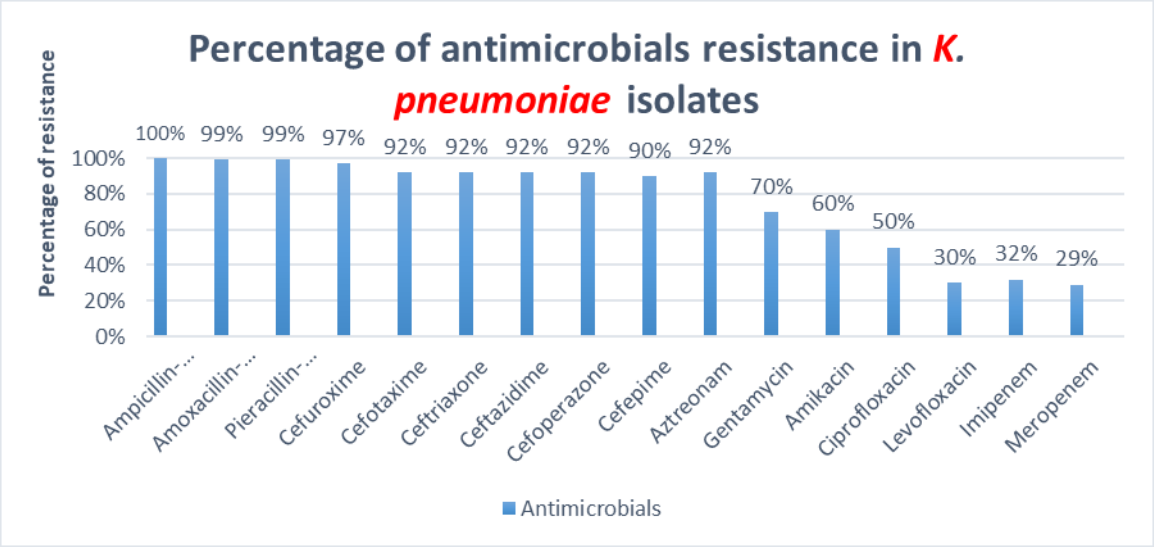


Figure 2. Prevalence of ESBL producers among *K. pneumoniae* isolates**.**

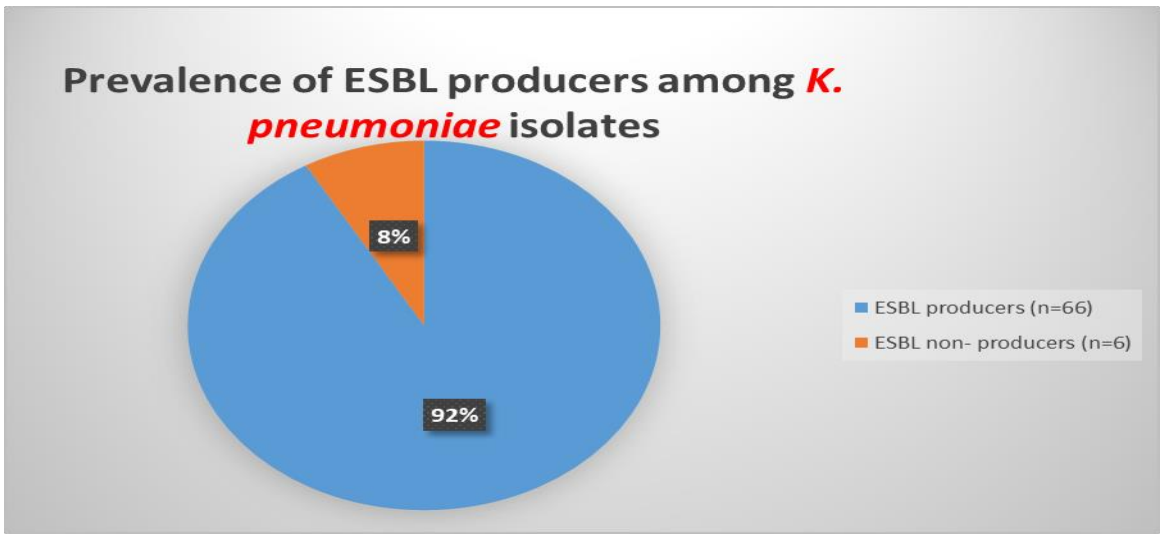
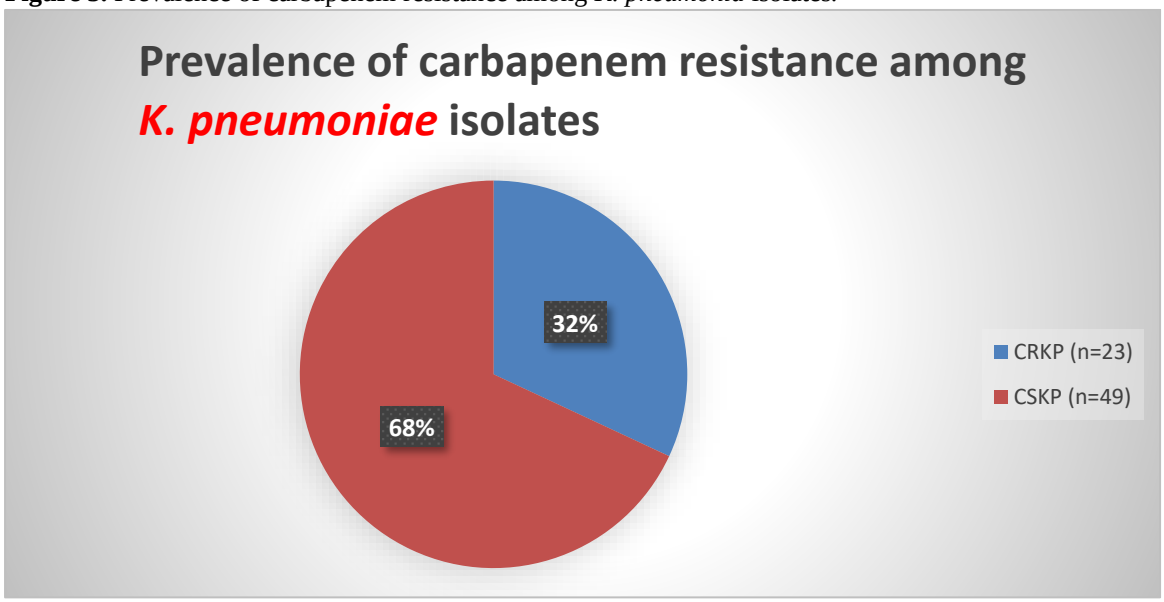


Figure 3. Prevalence of carbapenem resistance among *K. pneumonia* isolates.



Discsussion

There is a worldwide terrible increase in antimicrobial resistance to the commonly used antimicrobials. Continued surveillance for the bacterial resistance pattern is badly needed in order to ensure effective empirical therapy. To the best of our knowledge, our current study is the first study that highlights and determines the prevalence of CRKP bacteria in the NICU in our institute and it is also the first study that tested the in vitro activity of CAZ/ AVI against these bacteria in our hospitals.

In the present study, the number of confirmed neonatal sepsis was 136 cases with a percentage of (32.2%) concerning the total admitted cases. There was a wide variation in the rates of neonatal infections that have been reported in previous studies in Egypt and also worldwide. A study implemented in the same NICU in 2014 reported a relatively higher incidence rate of infection (38.5%), another study carried out in Benha university NICU in 2013 reported an incidence rate of (54%) and **Shitaye et al.** stated an infection rate of 44% in Ethiopia [21-23]. Lower incidence rates were reported in other NICUs reaching 21.4% and 14% in Egypt and Georgia respectively [24,25]. The differences among the studies can be attributed to the difference in surveillance time and methods used for detection of neonatal infections, variations in the study populations, the types of antibiotics used, and discrepancy in the awareness and adherence to infection control measures. Seventy-two (52.9%) Out of the 136 culture-proven cases of neonatal sepsis included in our study, were positive for *K. pneumoniae*, while the remaining 64 were positive for *Coagulase negative Staphylococci (n=24, 17.6%), E. coli, Acinetobacter, Staphylococcus aureus* were equally isolated with (n=10, 7.4%), *Pseudomonas aeruginosa (n=7, 5.1%)* and *Streptococcus pneumoniae (n=3, 2.2%). Klebsiella pneumoniae* was the most commonly isolated pathogen causing neonatal sepsis in our study and several studies from Egypt and different developing countries [22, 24, 26]. The increased incidence of *K. pneumoniae* infection could be explained by the following; its ability to thrive in the hospital setting and to spread between patients rapidly, its resistance to commonly used empiric antimicrobials, in addition to other risk factors in this age group like low birth weight, prematurity, and parenteral nutrition. On the contrary, other studies reported a predominance of Gram-positive organisms especially CONS [27,28]. This could be due to the excessive use of invasive devices for caring for preterm and LBW neonates especially in those NICU and also because the spectrum of different bacteria responsible for neonatal sepsis varies between NICUs in the same country and between different countries.

Extraordinary challenges are facing diagnosis and managing sepsis because of the undefinable signs and symptoms and the prolonged time of laboratory diagnosis, which lead to the continual prescription of empirical antimicrobial drugs to get rid of the suspected sepsis. Such practices contribute to the emergence of MDR, XDR, and PDR bacteria, diminishing the available therapeutic options, and reducing chances of recovery. In our study, we detected a great burden of drug-resistant *K. pneumoniae* at Zagazig University NICU. The antimicrobial resistance trends for the 72 isolates of *K. pneumoniae* bacteria was as follow; MDR (n=34, 47.2%), XDR (n=26, 36.1%). Unfortunately, the problem is not confined to our institute but many studies have reported the increase of MDR and XDR bacteria from Egypt and different areas of developing countries [29,17], many factors contribute to this increase like the Lack of regulations, over the counter sale of antibiotics, and bad hygienic conditions in the hospitals.

In our study, the highest resistance was detected with penicillins and cephalosporins with a resistance range (90%-100%). Previous reports in Egypt and other countries reported similar findings [30,31]. The resistance to ampicillin is intrinsic, while the high resistance to cephalosporin has resulted from the excess consumption of these antibiotics as the first and second lines of empirical treatment of neonatal sepsis in different NICUS. Resistance to aminoglycoside in our study was relatively high for gentamycin reaching 70% and for amikacin reaching 60%. This is similar to the results stated by **El-Din et al.**, who reported a close level of resistance to aminoglycosides, but a higher resistance level was detected by **Hassuna et al.**, who reported a resistance level of more than 95% [32,17]. The divergence in the resistance pattern to aminoglycoside is due to the variation in the type of aminoglycoside used in different NICUS. Our *K. pneumoniae* isolates were relatively susceptible to quinolones, as noticed by **Mohsen et al.** [33]. This relative sensitivity may be attributed to the limited use of quinolones in young children, as they may be

prescribed only in bacterial infections with resistance to other antibiotics guided by culture results.

The ESBL producers *K. pneumoniae* bacteria was extremely high among our isolates representing 92% of them. High levels (80%) were also reported by an Egyptian study by **Muhammed et al.** ; however, the percentage was lower in Myanmar in 2014 (38%) [34,35]. This high level of ESBL producers *K. pneumoniae* in our NICU could be explained by the overuse of empiric cephalosporins as empiric therapy leading to the selection of the ESBL strains and the ESBL genes are carried on a plasmid that could be easily transferred in between bacteria, spreading the resistance in non-resistant strains. Additionally, non-adherence of the medical staff to the infection control measures and non-cohorting of infants and staff increase the spread and exaggerate the problem.

We noted an incidence rate of CRKP (32%) among the isolated strains of *K. pneumoniae*, higher results were obtained by **Ghaith et al.** who reported a resistance rate of (56.5%), and an extremely high frequency of resistance was reported in another study reaching 95% [16,17]. This resistance pattern may be explained with the excess use of carbapenems antimicrobials for empiric treatment of neonatal sepsis that does not respond to penicillins and cephalosporins. On the contrary other similar study observed that *K.pneumoniae* was highly sensitive to meropenem due to the proper selection of cases for carbapenem treatment, which keep the sensitivity of these drugs [36].

The mechanism of resistance to carbapenems includes the production of enzymes called carbapenemases or a combination of structural mutations and the production of other β-lactamases. In our study, we tested the CRKP isolates (n=23) for the production of carbapenemases by mCIM and eCIM and we found that 100% of the isolates were carbapenemase producers, 39% (n=9) of them were serine carbapenemase producer, and the other 14 isolates (61%) were metallo-β lactamase producer. This finding goes in line with other studies that found that carbapenemases were the main resistance mechanism against carbapenems [5, 12].

Carbapenemase-producing *Enterobacteriaceae* (CPE) was defined by the U.S. Centers for Disease Control and Prevention (CDC) as the most elevated need for novel antimicrobial development in 2011. U.S. FDA-approved Ceftazidime-avibactam for the treatment of CPE. Administration of ceftazidime-avibactam appears to be well tolerated and efficacious without being associated with significant adverse events in infants and neonates [37]. This antibiotic is currently available in our country but has not been used yet in our hospitals. On testing the antibiotic disc of (CAZ/AVI) against our CRKP isolates we found that all the MBL producing strains were resistant to it (100% resistance), while the serine producing strains were highly resistant also with a resistance percentage of 77.8%. These findings were in partial agreement with previous studies which stated that most MBL-positive strains were resistant to CAZ-AVI, and the resistance rates ranged from 90.8% to 98.6% as avibactam is not effective against class B metallo-β lactamases [38]. On the other hand, other studies proved the efficacy of avibactam (AVI) against serine carbapenemases by reversibly acylate it [39]. However, the resistance rates reported about the serine carbapenemases producing CRKP by many studies ranged from (16.7% - 21%) which move away from our high level of resistance [38]. Many factors are possible and could explain the development of resistance against CAZ/AVI in our isolates e.g. loss of outer membrane proteins (OMPs), acquiring efflux pumps, changes in PBPs, beta-lactamase induction, presence of multiple (or even novel) beta-lactamases or mutation in the active site of β-lactamase, and Membrane impermeability[40].

We noticed that neonates with PROM, neutropenia, and CVC fixation had a significantly higher incidence of sepsis with CRKP bacteria than the other tested risk factors. These findings are partially consistent with **Akturk et al.** who found that neutropenia and invasive procedures were significant risk factors for CRKP infections. On the contrary, other studies reported prematurity, CS delivery and CVC fixation were the most significant risk factor for CRKP sepsis in neonates, any one of the previous risk factors represent a weak point for the bacteria to establish itself inside the immunocompromised neonate [5,16].

Despite great efforts to optimize antibiotic prescription and stewardship processes, bacterial resistance definitely cannot be stopped but only slowed down. Thereby, the continuous development of antimicrobials with an optimized spectrum of

activity is essential to meet the current needs and impending evolving resistance.

Conclusion and recommendations

The prevalence of CRKP is alarming in our NICU that necessitates continuous surveillance for the bacterial causes of neonatal sepsis. Ceftazidime/avibactam is an unsuitable option for the treatment of this type of resistant bacteria because of the high resistance against it. Further studies are needed on a larger number of bacteria for detection of the genetic bases of this resistance

Limitations of the study

Our study is one-center study and multi-center study is better to reflect the magnitude of CRKP in our locality. No molecular detection for the genes of resistance was done.

Conflicts of interest

No conflicts of interest present in our study.

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