

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/333092835>

# Emergence of AmpC beta-Lactamase-and Metallo-beta-Lactamase-producing *K. pneumoniae* in Sebha, Libya

Article · May 2019

---

CITATIONS

0

READS

243

1 author:



Khadija Mohamed Ahmad

Sebha University

22 PUBLICATIONS 20 CITATIONS

SEE PROFILE

## Emergence of AmpC $\beta$ -Lactamase- and Metallo- $\beta$ -Lactamase-producing *Klebsiella pneumoniae* in Sebha, Libya

Khadija Mohamed Ahmad<sup>1,2</sup>, Almahdi Ahmed Mohamed Alamen<sup>2</sup>, Shamsi Abdullah Mohamd Saad Shamsi<sup>3</sup>, Abdelkader A. Elzen<sup>3</sup>

<sup>1</sup>Department of Microbiology, Faculty of Medicine, Sebha University, Sebha, Libya

<sup>2</sup>Department of Microbiology, Sebha Medical Center, Sebha, Libya

<sup>3</sup>Department of Microbiology, Faculty of Science, Sebha University, Sebha, Libya

Corresponding author:

Khadija Mohamed Ahmad

Department of Microbiology, Faculty of Medicine, Sebha University, Department of Microbiology, Sebha Medical Center, P.O. Box 18758 Sebha, Libya

e-mail: kady\_sunrise@yahoo.com

### ABSTRACT

The emergence of AmpC  $\beta$ -lactamase- and metallo- $\beta$ -lactamase-producing Gram-negative bacteria has become a global concern. In the last 2 years, the resistance to broad-spectrum  $\beta$ -lactam antibiotics caused by *Klebsiella pneumoniae* has been noted among clinical isolates in Sebha Medical Center, Libya. During a period of 2 years, 22 nonrepetitive *K. pneumoniae* strains were obtained from neonates and identified in the microbiology unit. This study aimed to assess the antimicrobial resistance profile and phenotype of extended-spectrum  $\beta$ -lactamase (ESBL)- and AmpC  $\beta$ -lactamase-producing *K. pneumoniae* isolates. The antibiotic susceptibility of all isolates was evaluated according to the Clinical Laboratory Standards Institute guideline. Further screening using boric acid and ethylenediaminetetraacetate (EDTA) were performed to study metallo- $\beta$ -lactamase (MBL) and AmpC enzyme production. The study found that 100% of isolates were resistant to  $\beta$ -lactam group, and 91% were resistant to  $\beta$ -lactamase inhibitors. Moreover, 23% (5/24) of all isolates exhibited ESBL phenotype, and 59% (13/22) were AmpC enzyme producers. The resistance to other broad-spectrum antibiotics, for example, aminoglycosides and quinolones, was also observed in this study. This study reported the first case of carbapenem-resistant *K. pneumoniae* MBL in Sebha, in the south of Libya. This carbapenemase-producing strain exhibited remarkable resistance to cephalosporins, fluoroquinolones, and aminoglycosides. This study showed that AmpC and MBL screen tests were simple and could routinely be performed in the clinical laboratory using EDTA and boric acid.

**Key words:** AmpC enzyme, ESBL, *Klebsiella pneumoniae*, metallo- $\beta$ -lactamase, neonates, opportunistic organism

### INTRODUCTION

The emergence of antimicrobial resistance, especially to the  $\beta$ -lactam group, which is the most commonly used antibiotic to treat various infectious diseases, is particularly important because it limits the therapeutic options, thereby increasing the morbidity and mortality rates [1, 2]. In Enterobacteriaceae, antibiotic resistance is linked to different mechanisms, for example the production of a certain type of enzymes named as extended-spectrum  $\beta$ -lactamases (ESBLs). ESBLs are plasmid-mediated and efficiently hydrolyze penicillins, third-generation cephalosporins, and aztreonam, which are commonly used to treat infections [3, 4]. In addition, antibiotic resistance due to AmpC  $\beta$ -lactamases, 16S rRNA methylases, aminoglycoside-modifying enzymes, and carbapenemases has also been reported [5]. ESBL-producing

bacteria remain susceptible to carbapenems, and the activity of these enzymes is inhibited by clavulanic acid [6, 7].

In *K. pneumoniae*, AmpC beta-lactamases are located on plasmids, while in other Enterobacteriaceae spp., these enzymes are either plasmid or chromosomally encoded [8, 9]. AmpC  $\beta$ -lactamases confer resistance to cephamycins (e.g., cefoxitin and cefotetan) and  $\beta$ -lactamase inhibitor combinations [6]. Moreover, *K. pneumoniae* has also been found to harbor carbapenemase enzyme (KPC), which confers resistance to carbapenems such as imipenem and meropenem [10]. The emergence of carbapenem-resistant Enterobacteriaceae spp. in general and *K. pneumoniae*, in particular, has become a major public health problem due to lack of effective antibiotics [11], increasing the morbidity

and mortality rate especially in critically ill patients [12, 13]. Carbapenemases are usually expressed by plasmids or transposons, which can easily be transmitted to other bacteria via horizontal gene transfer [14]. Resistance to other groups of antibiotics has also been reported in Gram-negative bacilli [15,16]. *K. pneumoniae* are among the most frequently isolated opportunistic pathogens and found to be highly resistant to multiple antibiotics such as aminoglycosides, fluoroquinolones, and sulfonamides [17,18].

After 2011, multidrug-resistant *K. pneumoniae* strains were isolated from Libyan refugees in European hospitals [19, 20, 21] and also from a Libyan patient transferred to a Tunisian hospital [22].

Other studies also documented the emergence of ESBL-, AmpC enzyme-, and carbapenemase-producing bacteria in Libya. They isolated ESBL- and AmpC-positive *E. coli* and *K. pneumoniae* strains from patients admitted to Libyan hospitals in Tripoli, Zleiten, and El Khoms, Libya [23, 24].

Although ESBLs and AmpC  $\beta$ -lactamases have been discovered years ago, little is known about the presence of these enzymes in Libya in general and Sebha in particular. Therefore, the early detection of multidrug-resistant pathogens may help to avoid their transmission in the hospital and inappropriate antibiotic therapy.

This study aimed to determine the frequency of ESBL-, AmpC-, and carbapenemase-producing *K. pneumoniae* among hospitalized patients in the Department of Microbiology, Sebha Medical Center, Sebha, Libya, the biggest hospital in the south of Libya.

## MATERIALS AND METHODS

### Clinical isolates

All the clinical isolates were taken from neonates between January 2015 and January 2017. A total of 22 nonduplicate clinical isolates of *K. pneumoniae* were recovered. All these isolates were grown on MacConkey agar and 5% sheep blood agar medium (Oxoid, England) and then aerobically incubated overnight at 37°C. They were identified using the API20E system (bioMérieux, Marcy l'Etoile, France).

### Antimicrobial susceptibility testing

The antimicrobial susceptibility test was performed using the Kirby–Bauer disk diffusion method.

All isolates were tested for their susceptibility to antibiotics according to the Clinical Laboratory Standards Institute (CLSI) guideline [25]. Fresh colony (or colonies) was suspended in sterile water and streaked on Mueller–Hinton (MHA) agar (Oxoid) using McFarland 0.5 as control and incubated at 37°C for 16–18 h. The diameter of the inhibition zone for each antibiotic test was interpreted according to the CLSI recommendations [25]. The following antibiotics were used in this test: penicillin G (5  $\mu$ g), ampicillin (10  $\mu$ g), amoxicillin (20  $\mu$ g), augmentin (30  $\mu$ g), erythromycin (30 mg), gentamicin (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), ceftazidime (10  $\mu$ g), ceftriaxone (30  $\mu$ g), imipenem (10  $\mu$ g), nalidixic acid (30 mg), tetracycline (30  $\mu$ g), and chloramphenicol (30  $\mu$ g). They were supplied by Oxoid.

### Phenotypic detection of ESBLs using double disk synergy test

This study was performed according to the CLSI 2011 criteria [25]. The ESBL test was conducted when the organisms showed a zone of inhibition of <22 mm to ceftazidime or <23 mm to ceftriaxone. In this test, a suspension of a fresh colony was streaked on MHA (Oxoid). A combination of amoxicillin 20  $\mu$ g and clavulanic acid 10  $\mu$ g was placed centrally on the plate at 15-mm distance (center to center) from the disks containing ceftriaxone (30  $\mu$ g) and ceftazidime (10  $\mu$ g). The plates were incubated overnight at 37°C. An enhancement of the inhibition zone between the disks (ceftazidime/ceftriaxone) and clavulanic acid was considered as ESBL positive.

### Screening for AmpC $\beta$ -lactamase production using cefoxitin

The resistance to cefoxitin 30  $\mu$ g was used for the screening of AmpC  $\beta$ -lactamase producers in *K. pneumoniae* isolates. Based on the CLSI 2011 criteria [25], all isolates showing an inhibition zone of <18 mm were considered as AmpC  $\beta$ -lactamase producers and subjected to a confirmatory test using boric acid and ethylenediaminetetraacetate (EDTA).

### Phenotypic detection of AmpC $\beta$ -lactamase production

The AmpC enzyme production by *K. pneumoniae* was phenotypically detected using boric acid as a  $\beta$ -lactamase inhibitor, as recommended by Kiener in

1978 [26]. Boric acid (20  $\mu$ L) was dispensed onto commercially available cefoxitin disk. The disk was then left to dry at room temperature for 10 min. The agar plates (MHA with tested organism) were incubated at 37°C overnight. The diameter of the growth-inhibitory zone around the cefoxitin disk with boric acid was compared with that without boric acid. The test was considered positive when the diameter of the growth-inhibitory zone around cefoxitin with boric acid was >5 mm larger than that without boric acid. Based on the procedure proposed by Black in 2005 with a slight modification [27], EDTA was added to the cefoxitin disk immersed in boric acid to increase the permeability of bacterial cells, thus promoting the release of  $\beta$ -lactamases into the environment. Then, the zone of inhibition around the cefoxitin disk with and without the EDTA was compared.

#### Phenotypic detection of carbapenemase production

The strain showing resistance to imipenem was further subjected to carbapenemase production assay. In this assay, imipenem and imipenem/EDTA disks were used. Further, 10  $\mu$ L of EDTA (0.1M) was added to the imipenem disk and left to dry at room temperature for 10 min, as described by Yong 2002 [28]. The test organism suspension was adjusted to McFarland 0.5 standards and then inoculated on the Mueller–Hinton agar plate. Two imipenem disks, one with EDTA and the other without, were placed on the plate. The results of the two disks were compared after 16- to 18-h incubation at 37°C. An augmentation of the inhibition zone around imipenem with EDTA of at least 5 mm was considered as MBL positive.

## RESULTS

A total of 22 bacterial isolates were analyzed in this study. Regarding the  $\beta$ -lactam group, all the isolates were found to be resistant to penicillin, ampicillin, amoxicillin, and cephalexin, while the resistant to augmentin and ceftriaxone was 91% ( $n = 21$ ) and 82% ( $n = 18$ ), respectively. The resistance to gentamicin and ciprofloxacin was 77% and 4.5%, respectively, while three isolates showed an intermediate response. The results also showed that 95% ( $n = 21$ ) of the whole collection was resistant to erythromycin. Regarding the carbapenem group, only one iso-

late exhibited resistance to imipenem while the rest were sensitive. In addition, 4.5% were resistant to chloramphenicol while 14% were resistant to both tetracycline and nalidixic acid (Fig. 1). In this study, the majority of the strains were multidrug-resistant (data not shown). Out of 22 isolates, 18 were suspected to be ESBL producers and confirmed by double-disk synergy test (DDST). Only 5% were considered positive owing to the augmentation of the inhibition zone between ceftriaxone with clavulanic acid and ceftriaxone alone (Fig. 2A and 2B). Although the third-generation cephalosporins were used to detect ESBL production, the effect of ceftazidime and ceftriaxone as ESBL indicators was variable (Fig. 2A and 2B). The performance of ceftazidime in detecting ESBL was poor compared with ceftriaxone. In synergy with Augmentin, all ESBL-positive isolates showed enhancement of the inhibition zone with ceftriaxone but not with ceftazidime. Ceftriaxone, unfortunately, was not able to detect ESBL in 13 isolates. Indeed, the level of enzyme expression and the co-occurrence of other resistance mechanisms (AmpC enzymes, efflux, and altered porins) should be considered, which may result in different phenotypes among ESBL-positive isolates. The expression of AmpC enzymes was suspected in this study. Hence, strains ( $n = 13$ ) that could not be confirmed as ESBL positive were screened for AmpC  $\beta$ -lactamase enzyme production using the cefoxitin disk and confirmed using boric acid as an AmpC inhibitor. Boric acid alone could not confirm the expression of AmpC enzymes; however, when EDTA was added, the zone of inhibition around the cefoxitin disk with boric acid became obvious (Fig. 2D). The data showed that 12 out of 13 strains were AmpC producers and only one strain did not show any difference in the zone of inhibition around the cefoxitin disk with boric acid (inconclusive). Indeed, the inconclusive isolate needs further confirmation using a molecular technique to study its genotype.

In the present study, one strain was suspected to be a carbapenemase producer based on its resistance to imipenem. This imipenem-resistant strain was confirmed as MBL producer when the zone of inhibition around the imipenem disk increased >5 mm by adding EDTA compared with the imipenem disk alone (Fig. 2C). This imipenem-resistant isolate was resistant to all antibiotics used in this test.

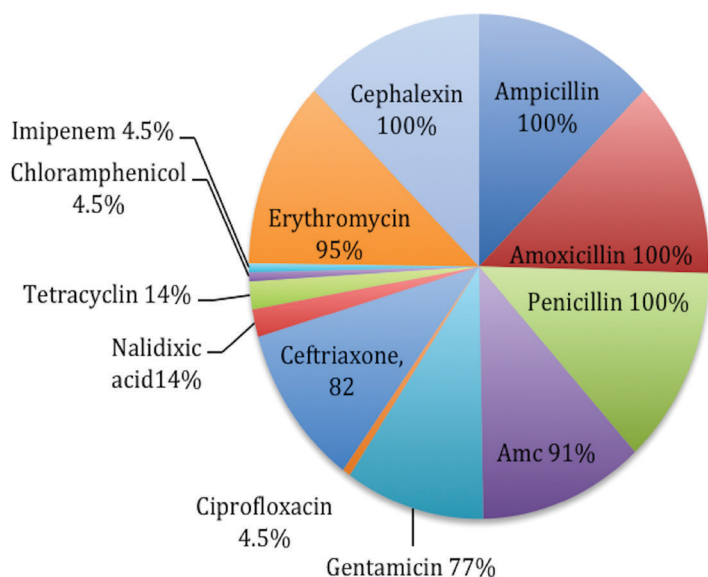


Figure 1: Antimicrobial resistance patterns of *K. pneumoniae* isolated from neonates at the Sebha Medical Center, Libya.

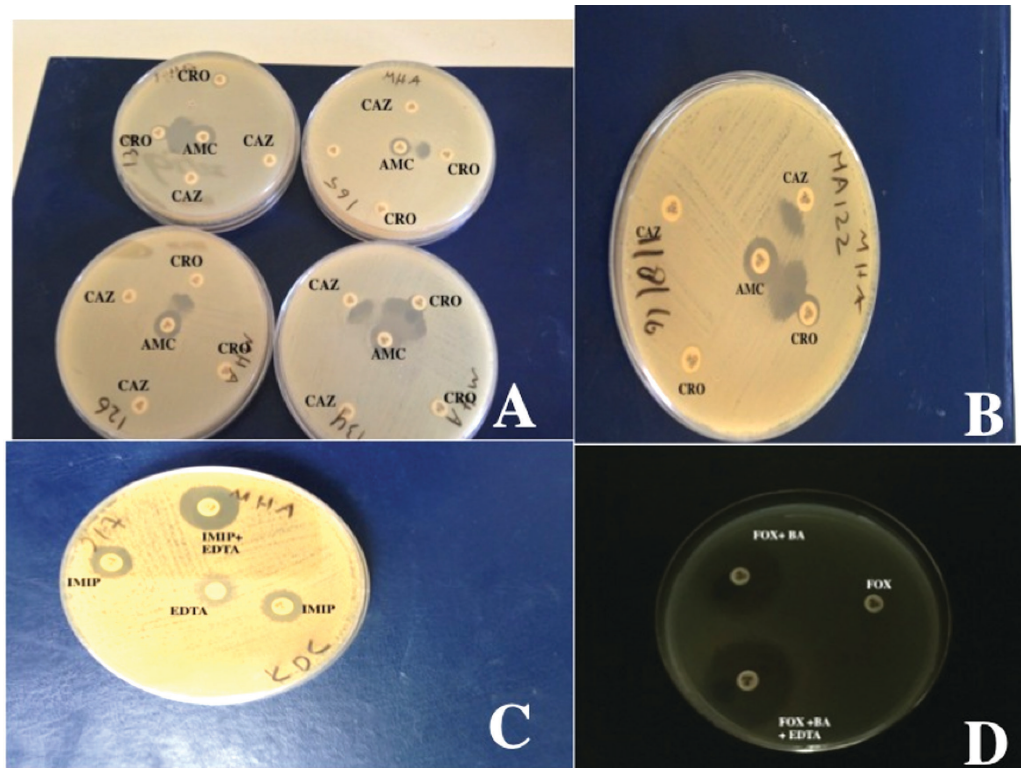
## DISCUSSION

The *K. pneumoniae* infection is an increasing threat to health with increased morbidity and mortality rates due to the lack of effective antibiotics. The present study focused on the neonate department and highlighted the propagation of nosocomial pathogens during a period of 2 years (2015–2017). The infections by pathogenic organisms can be transmitted in neonates through breast milk [29, 30]. Moreover, in health-care settings, the health-care workers serve as a carrier for the transmission of microorganisms through their hands or contaminated gloves [31, 32, 33, 34]. The results showed that the rate of resistance to the  $\beta$ -lactam antibiotic group (penicillin, ampicillin, and amoxicillin) was very high, which might be attributed to the misuse, overuse, or improper use of this class of antibiotics in the hospital or in the community setting. This finding supported the results achieved by Abujnah and his group in 2015 [35]. They reported that 100% of the *K. pneumoniae* isolates were resistant to ampicillin. This finding also confirmed the previously reported results [36, 37, 38, 39]. Further, resistance to first- and third-generation cephalosporins was also high (100% and 82% resistance to cephalexin and ceftriaxone, respectively). This finding was expected because both of these drugs were extensively used in the hospital and community settings in the Sebha city. The results of several studies agreed with the findings of the present study [40, 41, 42].

Quinolones are not commonly the first choice for neonatal infections in the Sebha Medical Center, owing to their low resistance rate (4.5%) to ciprofloxacin. However, Apondi in 2016 [43] and Molina in 2015 [44] reported different results; they showed that the resistance rate to quinolones was 41.3% and 55.5%–60.6%, respectively. Abujnah in 2015 [35] found that 17% of *K. pneumoniae* strains were resistant to quinolones.

In the Sebha Medical Center, the aminoglycosides were used as the first line of treatment for infections caused by Gram-negative bacteria. Therefore, the resistance to gentamicin (77%) was high. A similar finding was reported by another study performed in Egypt [45]. Gentamicin resistance has also been reported as high in other Libyan hospitals [46]. The resistance of *K. pneumoniae* to gentamicin was 75%. Further, Apondi and his colleagues reported a similar result in 2016 [43]. They found that 83% of all isolates were resistant to gentamicin. On the contrary, González [47] showed a different result: 58.07% of all isolates were sensitive to gentamicin.

The carbapenem group has been shown to be effective against nosocomial infections caused by Gram-negative bacteria. It is considered the last reliable treatment for bacterial infections so far. In the Sebha Medical Center, the use of carbapenems has increased in the last few years because of the resistance of bacteria to other commonly used  $\beta$ -lactam antibiotics. The resistance to imipenem was very low in the



**Figure 2:** ESBL, AmpC lactamase, and carbapenemase enzyme detection in *K. pneumoniae* isolates from neonates. (A and B) Double-disk synergy test (DDST) (phenotypic detection of ESBLs); the augmentation of the zone of inhibition between clavulanic acid (AMC) and ceftriaxone (CRO) or ceftazidime (CAZ) disks. (C) An increase in the zone of inhibition around the imipenem disk with EDTA (IMIP/EDTA) compared with imipenem (IMIP) alone [phenotypic detection of carbapenemase enzyme (MBL)]. (D) Phenotypic detection of AmpC lactamase enzymes; an increase in the zone of inhibition around cefoxitin (Fox) with boric acid (BA) compared with cefoxitin (FOX) alone. The inhibition zone became more obvious after adding EDTA

present study compared with that in other studies [48, 49]. Indeed, imipenem-resistant *K. pneumoniae* isolate in this study showed extensive resistance to the most commonly used antibiotics.

In the present study, ESBL-, AmpC-, and carbapenemase-producing *K. pneumoniae* isolated from neonates admitted to the Sebha Medical Center, Libya, were characterized. The prevalence of ESBL and AmpC enzymes has increased worldwide [50, 51, 52]. However, the dissemination of ESBL and AmpC enzymes has also been reported in the neighboring country Tunisia [21, 38, 53, 54, 55]. In Saudi Arabia, Kader and his group (2005) also isolated ESBL-producing uropathogens from both inpatients and outpatients. They also reported a high resistance of these organisms to different antimicrobial agents [36]. Recently, the spread of ESBL and AmpC enzymes has been documented in Libya. In 2017, Zorgani and his group found that 54% of *K. pneumoniae* isolates were ESBL producers [23]. Further, in Zleiten

and El Khoms, Libya, ESBL-producing *E. coli* was isolated from the fecal flora of pediatric age groups [24]. In the present study, ESBL enzymes were detected in 23% of all isolates and 59% were AmpC producers. Such coproduction of ESBL and AmpC enzymes was also reported by Zorgani in 2017. All AmpC enzyme producers were isolated from patients admitted to teaching hospitals in Tripoli, Libya [46]. Further, AmpC enzyme-producing *K. pneumoniae* isolates were also recovered from the pediatric department in Tripoli, Libya [23]. The prevalence of carbapenemase-producing *K. pneumoniae* has been recorded worldwide, including the Mediterranean region and North Africa [38, 21].

After Libyan conflict in 2011, *K. pneumoniae* isolates producing OXA-48 carbapenemase were recovered from patients transferred from Libya to Slovenia and Denmark [56, 20]. Moreover, OXA-48-producing *K. pneumoniae* isolates were isolated from patients admitted to Libyan hospitals [21]. This novel study

reported the emergence of AmpC enzyme- and carbapenemase-producing *K. pneumoniae* isolated from neonates admitted to the Sebha Medical Center, Libya.

The emergence of multidrug-resistant *K. pneumoniae* is alarming with a significant increase in the morbidity and mortality rates [57, 58]. In neonates with underdeveloped immunity, the prevalence of multidrug-resistant *K. pneumoniae* has been found as the main cause of neonatal infections especially in developing countries compared with developed countries [59, 60, 61, 62, 63, 64]. Further, the association between ESBL and AmpC enzymes produced by *K. pneumoniae* and multidrug resistance has been reported worldwide as the cause of mortality [65]. Although most of the isolates in this study showed resistance to multiple drugs, carbapenemase-producing *K. pneumoniae* exhibited resistance to all drugs. This finding was in agreement with other previously reported studies [60, 66]. Prolonged hospitalization and misuse and overuse of antibiotics were also considered risk factors, which increase the resistance rate among bacteria [63, 67]. However, this may be important especially in multidrug-resistant bacteria when the most common antibiotics are not effective, complicating the treatment of patients. In developing countries, the outspread of multidrug-resistant bacteria is considered as a clinical challenge because they are not usually detected using the routine susceptibility test and have a great potential to spread. The present study showed that the detection of ESBL, AmpC, and carbapenemase enzymes is not difficult and microbiologists can routinely do it to assess the outspread of multidrug-resistant bacteria, especially nosocomial pathogens. In developing countries where the molecular techniques are not always available, boric acid can help in detecting  $\beta$ -lactamase enzymes produced by nosocomial pathogens.

## REFERENCES

1. Vinod Kumar CS, Prasad BS, Kalapannavar NK, Raghu Kumar KG, Yogeeshha BK, Jayasimha VL, et al. Carbapenemases mediated resistance among the isolates of neonatal septicemia. *J Public Health Med Res.* 2013;1:24-7.
2. Chaudhary BN, Rodrigues C, Balaji V, Iyer R, Sekar U, Wattal C, et al. Incidence of ESBL producers amongst Gram-negative bacilli isolated from intra-abdominal infections across India (based on SMART study, 2007 data). *J Assoc Physicians India.* 2011;59:287-92.
3. Canton R., Novais A., Valverde A. Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. *Clin Microbiol Infect.* 14 (2008), pp. 144–153
4. Pałucha A, Mikiewicz B, Hryniewicz W, Gniadkowski M.J. Antimicrob Chemother. Concurrent outbreaks of extended-spectrum beta-lactamase-producing organisms of the family Enterobacteriaceae in a Warsaw hospital. 1999 Oct;44(4):489-99.
5. Roy S, Datta S, Viswanathan R, Singh AK, Basu S. Tigecycline susceptibility in *Klebsiella pneumoniae* and *Escherichia coli* causing neonatal septicemia (2007-10) and role of an efflux pump in tigecycline non-susceptibility. *J Antimicrob Chemother.* 2013;68:1036-42.
6. Bush K1, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother.* 1995 Jun;39(6):1211-33.

## CONCLUSIONS

A periodic antibiogram study should be performed to limit the progress of multidrug resistance. This study highlighted the pattern of antibiotic susceptibility and prevalence of multidrug resistance among Gram-negative pathogens most likely acquired during patient stay in the hospital. Health-care workers should routinely be screened for the presence of multidrug-resistant bacteria, and their education regarding hand hygiene and convenient aseptic techniques should be encouraged. Further, appropriate and effective good policies and drugs should always be considered to avoid the spread of multidrug-resistant bacteria especially among immunosuppressed patients.

## DECLARATIONS

### Availability of supporting data

All data generated or analyzed during this study are included in this manuscript; some supplementary files will be available from the corresponding author on request.

### Competing interests

The authors declare no competing interests.

### Funding

No funding was received for this study.

## ACKNOWLEDGMENT

The authors would like to thank the laboratory staff at the Department of Microbiology, Sebha Medical Center, and the Department of Microbiology, Sebha University, for their help and cooperation.

7. Bush K and Jacoby GA. Updated Functional Classification of  $\beta$ -Lactamases. *ANTIMICROBIAL AGENTS AND CHEMOTHERAPY*, Mar. 2010, p. 969–976.
8. Livermore, D. M.  $\beta$ -Lactamases in laboratory and clinical resistance. *Clin. Microbiol. Rev.* 1995. 8:557–584.
9. Thomson KS. Controversies about extended spectrum and AmpC  $\beta$ -lactamases. *Emerg Infect Dis.* 2001. 7:333–6.
10. Livermore D M. Bacterial resistance to carbapenems. *Adv Exp Med Biol.* 1995; 390:25–47.
11. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria, *Lancet Infect Dis.* 2009. vol. 9 (pg. 228-36)
12. Marchaim D, Navon-Venezia S, Schwaber MJ, Carmeli Y. Isolation of imipenem-resistant *Enterobacter* species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes, *Antimicrob Agents Chemother.* 2008. vol. 52 (pg. 1413-8).
13. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies, *Infect Control Hosp Epidemiol.* 2008. vol. 29 (pg. 1099-106)
14. Arnold RS, Thom KA, Sharma S, et al. Emergence of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *South Med J* 2011; 104:40–5.
15. Paterson DL, Bonomo RA. Extended-spectrum  $\beta$ -lactamases: a clinical update. *Clin Microbiol Rev.* 2005; 18:657–86.
16. Kaur J, Mahajan G., Chand K., Sheevani, Chopra S. Enhancing Phenotypic Detection of ESBL in AmpC co-producers by using Cefepime and Tazobactam. *Journal of Clinical and Diagnostic Research.* 2016. Vol-10 (1).
17. Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol.* 1998. Rev 11: 589–603.
18. Bradford PA, Bratu S, Urban C, Visalli M, Mariano N, Landman D, Rahal JJ, Brooks S, Cebular S, Quale J. Emergence of Carbapenem-Resistant *Klebsiella* Species Possessing the Class A Carbapenem-Hydrolyzing KPC-2 and Inhibitor-Resistant. *Clin Infect Dis.* 2004 Jul 1;39(1):55-60.
19. Kocsis E, Savio C, Piccoli M, Cornaglia G, Mazzariol A. *Klebsiella pneumoniae* harbouring OXA-48 carbapenemase in a Libyan refugee in Italy. *Clin Microbiol Infect.* 2013. 19(9):E409-11.
20. Hammerum AM, Larsen AR, Hansen F, Justesen US, Friis-Møller A, Lemming LE, Fuursted K, Littauer P, Schønning K, Gahrn-Hansen B, Ellermann-Eriksen S, Kristensen B. Patients transferred from Libya to Denmark carried OXA-48-producing *Klebsiella pneumoniae*, NDM-1-producing *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Agents.* 2012. 40(2):191-2
21. Mathlouthi N, Al-Bayssari C, El Salabi A, Bakour S, Ben Gwief S, Zorgani AA, Jridi Y, Ben Slama K, Rolain JM, Chouchani C. 2016. Carbapenemases and extended-spectrum  $\beta$ -lactamases producing *Enterobacteriaceae* isolated from Tunisian and Libyan hospitals. *J Infect Dev Ctries.* 2016 Aug 2;10(7):718-27.
22. Ben Nasr A, Decré D, Compain F, Genel N, Barguelli F, Arlet G. 2013. Emergence of NDM-1 in association with OXA-48 in *Klebsiella pneumoniae* from Tunisia. *Antimicrob Agents Chemother.* 257(8):4089-90.
23. Zorgani A., Elahmer O., Bashein A., Hawas A., Aljerbi A., Ziglam H., Elzouki A. 2017. Extended-Spectrum Beta-Lactamase- and Carbapenemase-Producing *Enterobacteriaceae* among Libyan Children. *EC Microbiology.* 2017. 126-135
24. Ahmed SF et al. *Ann Clin Microbiol Antimicrob.* Fecal carriage of extended-spectrum  $\beta$ -lactamases and AmpC-producing *Escherichia coli* in a Libyan community. *Ann Clin Microbiol Antimicrob.* 2014 Jun 16;13:22.
25. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement CLSI document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute. 2011. 31:M100-S21.
26. Kiener PA, Waley SG. Reversible inhibitors of penicillinases. *Biochem J.* 1978 Jan 1;169(1):197-204.
27. Black, J. A., E. S. Moland, and K. S. Thomson. AmpC disk test for detection of plasmid-mediated AmpC  $\beta$ -lactamases in *Enterobacteriaceae* lacking chromosomal AmpC  $\beta$ -lactamases. *J. Clin. Microbiol.* 2005. 43:3110– 3113.
28. Yong D1, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo- $\beta$ -lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol.* 2002. 40(10): 3798-801.
29. Godambe S, Shah PS, Shah V. Breast milk as a source of late onset neonatal sepsis. *Pediatr Infect Dis J.* 2005. Apr; 24(4):381-2.
30. Widger J, O'Connell NH, Stack T. Breast milk causing neonatal sepsis and death. *Clin Microbiol Infect.* 2010. 16(12):1796-8.
31. Gupta A, Della-Latta P, Todd B, San Gabriel P, Haas J, Wu F, Rubenstein D, Saiman L. Outbreak of extended-spectrum  $\beta$ -lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit linked to artificial nails. *Infect Control Hosp Epidemiol.* 2004. 25(3):210-5.
32. Lin R, Wu B, Xu XF, Liu XC, Ye H, Ye GY. Extended-spectrum  $\beta$ -lactamase-producing *Klebsiella pneumoniae* infection in a neonatal intensive care unit. *World J Pediatr.* 2012. 8(3):268-71.
33. Rock C, Thom K. A, Masnick, Johnson J.K, Harris A. D, and Morgan D. J. Frequency of *Klebsiella pneumoniae* carbapenemase (KPC) and non-KPC-producing *Klebsiella* contamination of Healthcare workers and the environment. *Infect Control Hosp Epidemiol.* 2014. 35(4): 426–429.
34. Rock C, Kerri A. Thom, Max Masnick, J. Johnson K, Anthony D. Harris, Daniel J Morgan.. Frequency of *Klebsiella pneumoniae* carbapenemase (KPC) and non-KPC-producing *Klebsiella* contamination of Healthcare workers and the environment. *Infect Control Hosp Epidemiol.* 2015. 35(4): 426–429
35. Abujnah AA, Zorgani A, Sabri MA, El-Mohammady H, Khalek RA, Ghenghesh KS. Multidrug resistance and extended-spectrum  $\beta$ -lactamases genes among *Escherichia coli* from patients with urinary tract infections in Northwestern Libya. *Libyan J Med.* 2015. 10: 26412.
36. Kader AA, Angamuthu K. Extended-spectrum  $\beta$ -lactamases in urinary isolates of *Escherichia coli*, *Klebsiella pneumoniae* and other gram-negative bacteria in a hospital in Eastern Province, Saudi Arabia. *Saudi Med J.* 2005 Jun;26(6):956-9.
37. Ko WC, Hsueh PR. Increasing extended-spectrum  $\beta$ -lactamase production and quinolone resistance among Gram-negative bacilli causing intra-abdominal infections in the Asia/Pacific region: data from the Smart Study 2002-2006. *J Infect.* 2009 Aug; 59(2):95-103.
38. Chouchani C, Marrakchi R, El Salabi A. Evolution of  $\beta$ -lactams resistance in Gram-negative bacteria in Tunisia. *Crit Rev Microbiol.* 2011. 37: 167-177.
39. N G, Girish C Math, Kavita Nagshetty, Shripad A Patil, Subhashchandra M Gaddad, Channappa T Shivannavar. Antibiotic Susceptibility Pattern of ES $\beta$ L Producing *Klebsiella pneumoniae* Isolated from Urine Samples of Pregnant Women in Karnataka. *J Clin Diagn Res.* 2014. 8(10): DC08–DC11.
40. Mansury D, Motamedifar M, Sarvari J, Shirazi B, Khaledi A. Antibiotic susceptibility pattern and identification of extended spectrum  $\beta$ -lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* from Shiraz, Iran. *J Microbiol.* 2016 Feb;8(1):55-61



41. Jain M, Varghese J, Michael T, Kedarishetty CK, GB, Swaminathan S, Venkataraman J. An Insight into Antibiotic Resistance to Bacterial Infection in Chronic Liver Disease. *J Clin Exp Hepatol*. 2017. 7(4):305-309.
42. Remschmidt C, Schneider S, Meyer E, Schroeren-Boersch B, Gastmeier P, Schwab F. Dtsch Arztebl. Surveillance of Antibiotic Use and Resistance in Intensive Care Units (SARI). *Int*. 2017. 114(50):858-865. doi:
43. Apondi OE, Oduor OC, Gye BK, Kipkoech MK. HIGH PREVALENCE OF MULTI-DRUG RESISTANT *KLEBSIELLA PNEUMONIAE* IN A TERTIARY TEACHING HOSPITAL IN WESTERN KENYA. *Afr J Infect Dis*. 2016. 10(2):89-95.
44. Molina-Lopez J, Aparicio-Ozores G, Ribas-Aparicio RM, Gavilanes-Parra S, Chavez-Berocal ME, Hernandez-Castro R, et al. Drug resistance, serotypes, and phylogenetic groups among uropathogenic *Escherichia coli* including O25-ST131 in Mexico City. *J Infect Dev Ctries*. 2011; 5: 8409.
45. El-Badawy MF, Tawakol WM, El-Far SW, Maghrabi IA, Al-Ghamdi SA, Mansy MS, Ashour MS, Shohayeb MM. Molecular Identification of Aminoglycoside-Modifying Enzymes and Plasmid-Mediated Quinolone Resistance Genes among *Klebsiella pneumoniae* Clinical Isolates Recovered from Egyptian Patients. *Int J Microbiol*; 2017:8050432.
46. Zorgani A, Daw H, Sufya N, Bashein A, Elahmer O, Chouchani C. Co-Occurrence of Plasmid-Mediated AmpC  $\beta$ -Lactamase Activity Among *Klebsiella pneumoniae* and *Escherichia Coli*. *Open Microbiol J*. 2017. 11:195-202.
47. González Mesa L, Ramos Morí A, Nadal Becerra L, Morffi Figueroa J, Hernández Robledo E, Alvarez AB, Marchena Bequer JJ, González Alemán M, Villain Plous C. Phenotypic and molecular identification of extended-spectrum beta-lactamase (ESBL) TEM and SHV produced by clinical isolates *Escherichia coli* and *Klebsiella* spp. in hospitals. *Rev Cubana Med Trop*. 2007. 59(1):52-8. Spanish.
48. Sun O. Park, Jianfang Liu, E. Yoko Furuya, Elaine L. Larson. Carbapenem-Resistant *Klebsiella pneumoniae* Infection in Three New York City Hospitals Trended Downwards From 2006 to 2014. *Open Forum Infect Dis*. 2016. 3(4): ofw222.
49. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT, et al. European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis*. 2017. 17(2):153-163.
50. Varsha Gupta, Karthikeyan Kumarasamy, Neelam Gulati, Ritu Garg, Padma Krishnan, Jagdish Chander. 2012. AmpC  $\beta$ -lactamases in nosocomial isolates of *Klebsiella pneumoniae* from India. *Indian J Med Res*. 2012 Aug; 136(2): 237–241.
51. Soltan Dallal MM, Sabbaghi A, Molla Agha H, et al. Prevalence of AmpC and SHV  $\beta$ -lactamases in clinical isolates of *Escherichia coli* from Tehran hospitals. *Jundishapur J Microbiol*. 2013. 6:176-80.
52. Izzati F, Khari M., Karunakaran R., Rosli R., Tay S. T. Genotypic and Phenotypic Detection of AmpC  $\beta$ -lactamases in Enterobacter spp. Isolated from a Teaching Hospital in Malaysia. *PLoS One*. 2016. 11(3): e0150643.
53. Chouchani C, Ben AN, M'Charek A, Belhadj O. First characterization in Tunisia of a TEM-15, extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolate. *Microb Drug Resist*. 2007. 13: 114-118.
54. Ben AN, Mercuri PS, Ben MM, Galleni M, Belhadj O. Characterization of a novel extended-spectrum TEM-type beta-lactamase, TEM-164, in a clinical strain of *Klebsiella pneumoniae* in Tunisia. *Microb Drug Resist*. 2009. 15: 195-199.
55. Yaici L, Haenni M, Métayer V, Saras E, Mesbah Zekar F, Ayad M, Touati A, Madec JY.. Spread of ESBL/AmpC-producing *Escherichia coli* and *Klebsiella pneumoniae* in the community through ready-to-eat sandwiches in Algeria. *Int J Food Microbiol*. 2017. 20;245:66-72.
56. Pirš M, Andlovic A, Cerar T, Žohar-Čretnik T, Kobola L, Kolman J, Frelih T, Prešern-Štrukelj M, Ružič-Sabljčić E, Seme K. A case of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* in a patient transferred to Slovenia from Libya, November 2011. *Euro Surveill*. 2011 Dec 15;16(50):20042.
57. Patricia AB, Simona B, Carl U, Melissa V, Noriel M., David L. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30  $\beta$ -lactamases in New York City. *Clin Infect Dis*. (2004). 39, 55–60.
58. Lee K, Lee MA, Lee CH, Lee J, Roh KH, Kim S. Increase of ceftazidime- and fluoroquinolone-resistant *Klebsiella pneumoniae* and imipenem-resistant *Acinetobacter* spp. in Korea: analysis of KONSAR study data from 2005 and 2007. *Yonsei Med J*. 2010. 51(6), 901-911.
59. Vergnano S., Sharland M., Kazembe P., Wansambo C.M., Heath PT Neonatal sepsis, An international perspective, *Arch. Dis. Child*. 2005. Fetal Neonatal Ed., 90: F220-F224
60. Muley VA, Ghadage DP, Bhore AV. Bacteriological Profile of Neonatal Septicemia in a Tertiary Care Hospital from Western India. *J Glob Infect Dis*. 2015. 7(2):75-7.
61. Al-Rabea AA1, Burwen DR, Eldeen MA, Fontaine RE, Tenover F, Jarvis WR. *Klebsiella pneumoniae* bloodstream infections in neonates in a hospital in the Kingdom of Saudi Arabia. *Infect Control Hosp Epidemiol*. 1998 Sep;19(9):674-9.
62. Gupta A. Hospital-acquired infections in the neonatal intensive care unit—*Klebsiella pneumoniae*. *Semin Perinatol*. 2002 Oct;26(5):340-5.
63. Desimoni MC1, Esquivel GP, Merino LA. Fecal colonization by extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit]. *Enferm Infect Microbiol Clin*. 2004. 22(9): 507-11.
64. Dashti AA, Jadaon MM, Gomaa HH, Noronha B, Udo EE. Transmission of a *Klebsiella pneumoniae* clone harbouring genes for CTX-M-15-like and SHV-112 enzymes in a neonatal intensive care unit of a Kuwaiti hospital. *J Med Microbiol*. 2010. 59(Pt 6):687-92.
65. El Nekidy WS, Mooty MY, Attallah N, Cardona L, Bonilla MF, Ghazi IM. Successful treatment of multidrug resistant *Klebsiella pneumoniae* using dual carbapenem regimen in immunocompromised patient. *IDCases*. 2017. 15; 9:53-55.
66. Brady M, Cunney R, Murchan S, Oza A, Burns K. *Klebsiella pneumoniae* bloodstream infection, antimicrobial resistance and consumption trends in Ireland: 2008 to 2013. *Eur J Clin Microbiol Infect*. 2016. 35(11):1777-1785.
67. Haji H. B, Farzanehkhah M, Dolatyar A, Imani M, Farzami MR, Rahbar M, et al. A study on prevalence of KPC producing from *Klebsiella pneumoniae* using Modified Hodge Test and CHROMagar in Iran. *Ann Biol Res*. 2012. 3(12): 5659-64