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ORIGINAL ARTICLE

Insight on the Prevalence of Clinical *Klebsiella* Isolates Producing Extended Spectrum Beta-Lactamases

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ABSTRACT

Background: *Antimicrobial resistance is increasing, particularly in Gram-negative bacteria such as Klebsiella species, which can cause acute infections and complications in intensive care units.* ***Objectives:*** *This study aims to investigate antimicrobial susceptibility patterns and the frequency of resistance among clinical Klebsiella isolates, focusing on prevalence of extended spectrum β-lactamases.* ***Methodology:*** *One hundred and eighty-one clinical isolates of Klebsiella spp. obtained from the Kasr Al Ainy Hospital's Microbiological Laboratorywere subjected to phenotypic identification and antibiotics susceptibility testing using the Vitek 2 system.* ***Results:*** *115 isolates out of 181 were identified as Klebsiella species. Of which, K. pneumoniae represented 89% while K. ozaenae and K. oxytoca were 7 and 4%, respectively. Only 53 isolates displayed extended spectrum β-lactamases (ESBL) Klebsiella. Throughout the susceptibility test of thirteen antibiotics, the highest resistant pattern (100%) was observed against ampicillin. Furthermore, ESBL Klebsiella spp. exhibited high resistance against cefotaxime, amikacin, and ciprofloxacin.* ***Conclusion:*** *This investigation revealed that K. pneumoniae subsp. pneumoniae was the dominant followed by K. pneumoniae subsp. ozaenae, and K. oxytoca. The highest resistance was identified among ESBL-producing clinical isolates; also, our results indicate extended drug resistance among non-ESBL-producing isolates.*

INTRODUCTION

The emergence of antimicrobial resistance among microorganisms is a serious public health concern and extended-spectrum β-lactamases (ESBLs)-producing *Enterobacterales* is one of the major concerns among antibiotic-resistant bacteria. The prevalence of ESBL in *Enterobacterales* has been increasing with time, and it differs according to the species, hospital allocation, sources of infections, nosocomial or community acquisitions, and geographic regions.[1] The World Health Organization (WHO) has identified the third and fourth generation cephalosporins and carbapenems as antibiotics that are critically important due to, they're last resort for treating antimicrobial-resistant bacteria. However, recent reports of resistance have increased.[2] The WHO's Global Action Plan aims to optimize antimicrobial use through improved diagnostics and surveillance.[3]

Klebsiella is a rod-shaped, gram-negative bacterium which is present in the intestines, skin, and mouth. It can lead to nosocomial and community infections and, via ESBLs, can become resistant to β-lactam medicines.[4,5] *Klebsiella* species can lead to wound infections, septicemia, pneumonia, and urinary tract infections. Since the discovery of antibiotics in 1928, fluctuations in incidence and prevalence have made treatment a therapeutic challenge.[6] The most isolated *Klebsiella* spp. in human clinical samples is *Klebsiella pneumoniae*, one of the genus' important species.[5] *K. pneumoniae* is the most common bacterial species that produces ESBLs.[7] Since its discovery in 1882, *K. pneumoniae* has been associated with several multidrug-resistant Gram-negative infections that have a significant detrimental socioeconomic impact and increase hospitalizations, morbidity, and death.[8,9]

Globally, *K. pneumoniae* is one of the six leading antibiotic-resistant bacterial pathogens causing over 71% of deaths due to antimicrobial resistance.[10] It is a well-established fact that developing resistance to one antibiotic can confidently lead to resistance to other antibiotics, significantly limiting treatment options and rising mortality rates.[11,12] Also, Russo and Marr mentioned that hypervirulent *K. pneumoniae*, more virulent than classical, primarily infects healthy people in the Asian Pacific Rim, necessitating rapid detection and treatment due to its increased risk of endophthalmitis and central nervous system infections.[13] It has been pointed out that surveillance systems are necessary to monitor bacterial changes and antibiotic resistance patterns since drug-resistant *K. pneumoniae* isolates in Egypt have limited healthcare treatment choices. Therefore, this research aims to investigate antimicrobial susceptibility patterns and the frequency

of resistance, focusing on ESBLs prevalence among clinical *Klebsiella* isolates.

METHODOLOGY

Media used for purification and identification were purchased from Oxoid, England. However, Tryptic Soy, Muller Hinton agar & broth were purchased from (Lab M, England) and Dimethyl Sulfoxide (Tedia, USA). The ethical approval was obtained from the Egyptian Drug Authority (EDA), Giza, Egypt.

Antibiotics used for susceptibility test of *Klebsiella* species:

Two reference strains, *Escherichia coli* (*E. coli* ATCC 25922) and *Klebsiella pneumoniae* (*K. pneumoniae* ESBL ATCC 700603) were obtained from the American Type Culture Collection.

All antibiotic discs purchased from Oxoid company however, ceftazidime, cefotaxime, ceftazidime/Clavulanic, and cefotaxime/clavulanic purchased from Hi media, India. Microdilution indicator of 2,3,5-triphenyl tetrazolium chloride (TTC) was purchased from research lab fine chemical industries, India.

Phenotypic identification of bacterial isolates:

Clinical isolates thought to belong to a *Klebsiella* species were obtained from the Kasr Al Ainy Hospital's Microbiological Laboratory, Egypt. Primary culture was conducted on the MacConkey agar for lactose fermentation. Biochemical conventional tests were used including: urease test, motility, and gas production; IMViC tests including four reaction tests (Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test) according to the standard protocols. The identification of *Klebsiella* species was further confirmed by VITEK II system (BioMèrieux, France) at Animal Health Research Institute. All *Klebsiella* spp.isolates were preserved in 20% tryptone toy broth /glycerol Eppendorf's at -20 ºC for further studies.

Susceptibility test for antibiotic resistance of *Klebsiella* isolates:

Applying the Kirby Bauer Disk Diffusion technique on Muller Hinton (MH) agar entirely covered by prepared 0.5 McFarland bacterial solution yielding a solution of 1 x 10^7 CFU/ml. Thirteen antibiotic discs were used in the antimicrobial susceptibility test as follow: ampicillin (10µg), ampicillin/sulbactam (10/10µg), imipenem (10µg), ceftazidime (30µg), cefotaxime(30µg), ceftriaxone (30 µg), cefuroxime (30µg), amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg), norfloxacin (10µg), chloramphenicol (30µg) and doxycycline (30µg) to determine the sensitivity pattern of *Klebsiella* isolates. The quality control ranges of reference strain *E. coli* ATCC (25922) were employed as a positive control per the CLSI guidelines. Each plate was covered by antimicrobial disks, incubated at 37 °C for 24 h. Antibiotic discs and inhibition zone diameters interpretative were classified as susceptible, moderate, or resistant as provided by the CLSI M100-A9 (CLSI, 2020).[14]

Screening for ESBLs production:

Using the Kirby Bauer disk diffusion technique, all collected *Klebsiella* spp. were screened for the formation of ESBLs via cefotaxime (30µg), cefotaxime/clavulanic acid (30/10µg), ceftazidime (30µg), and ceftazidime/Clavulanic (30/10µg). The reference *K. pneumoniae* (ATCC 700603) was utilized as a positive control. Samples were cultured on MH agar plates, with antibiotic discs spaced 25 mm apart. After 18 h of incubation at 37 °C, each plate was examined, and the diameters of the inhibitory zones were calculated. Isolates that showed ≥ 5 mm increase in zone diameter of ceftazidime/clavulanic than ceftazidime alone in addition increase in zone diameter ≥ 3 mm of cefotaxime/clavulanic acid than cefotaxime alone considered as an ESBL strains (CLSI, 2020).

Determination of minimum inhibitory concentrations (MICs) of β-lactam antibiotics against most resistant *Klebsiella* spp. producing ESBLs:

MICs were established through broth microdilution in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, and the interpretation utilized the 2020 CLSI. For the resistant ESBL-producing isolates that were chosen for continuing the study, MICs were determined using cefotaxime, ceftazidime, and ceftriaxone. As per the CLSI guidelines, *Escherichia coli* ATCC 25922 was utilized as the reference strain. The TTC indicator dye was applied to ascertain the MIC end point. The dye is colorless, changed to red as an indication of bacterial growth.

RESULTS

In a year and a half, 181 suspected *Klebsiella* isolates were collected from hospitalized patients in the ICU, Chest, and Urology units at Kasr El Ainy Educational Hospital. Clinical specimens (blood culture, endotracheal and sputum culture, urinary tract infection (UTI) specimen, and wound swabs). Only 115 (64%) of bacterial isolates showed typical *Klebsiella* species characteristics, including Gram-negative, short rod-shaped rose colonies on MacConkey agar, and growth on selective and differential media, as confirmed by microscopic and biochemical tests.

According to the biochemical examination, the samples were identified into several species. Then, results were confirmed by VITEK 2 analysis. The highest prevalence species was *K. pneumoniae* subsp. pneumoniae (n= 102) representing 89%. However, *K. pneumoniae* subsp. *ozaenae* was (n = 8) revealing 7%

followed by *K. oxytoca*, (n= 5) with 4% out of 115 total isolates.

Table (1) illustrates *Klebsiella* spp. resistance rates among eight antibiotic classes. The result shows that ampicillin, ampicillin/sulbactam and ceftriaxone, were the antibiotics with the highest incidence of resistance, as reported by 100, 89, and 80% of respondents, respectively. Conversely, the antibiotics with the least amount of resistance were shown in Imipenem and amikacin (44%) per each, followed by gentamicin (39%), and chloramphenicol (32%). Also, ESBL-producing *Klebsiella* spp. were identified in 53 resistant species which represent in (46%) out of 115 isolates by using zone diameter breakpoints, which provide interpretative standards for CLSI recommendations.

Table 1: Antimicrobial susceptibility patterns conducted on 115 *Klebsiella* spp.

Antibiotic Abb. µg/disc	Antibiotic Name	Antibiotic Class	S		I		R	
			No.	%	No.	%	No.	%
CIP 5	Ciprofloxacin	Fluoroquinolone	35	**30**	2	**2**	78	**68**
SAM 10/10	Ampicillin/Sulbactam	Penicillins	9	**8**	4	**3**	102	**89**
CRO 30	Ceftriaxone	3rd generation Cephalosporin	22	**19**	1	**1**	92	**80**
NOR 10	Norfloxacin	Fluoroquinolone	36	**31**	1	**1**	78	**68**
CXM 30	Cefuroxime	2nd generation Cephalosporin	11	**10**	14	**12**	90	**78**
C 30	Chloramphenicol	Phenicols	75	**66**	3	**2**	37	**32**
AK 30	Amikacin	Aminoglycoside	56	**49**	9	**8**	50	**44**
CN 10	Gentamicin	Aminoglycoside	62	**54**	8	**7**	45	**39**
IMP 10	Imipenem	Carbapenems	60	**52**	5	**4**	50	**44**
DO 30	Doxycycline	Tetracycline	33	**29**	12	**10**	70	**61**
AMP 10	Ampicillin	Penicillins	0	**0**	0	**0**	115	**100**
CAZ 30	Ceftazidime	3rd generation Cephalosporin	19	**13**	10	**9**	85	**74**
CTX 30	Cefotaxime	3rd generation Cephalosporin	12	**10**	2	**2**	90	**78**

S: susceptible **I:** intermediate **R**: resistant

ESBL phenotype confirmation test *Klebsiella* spp. was conducted throughout 53 ESBL-producing *Klebsiella* spp. against ceftazidime and cefotaxime alone and in combined with clavulanic acid. Our results illustrate that fifteen isolates demonstrate the highest resistance as ESBL *Klebsiella* spp., Table (2).

Table 2: ESBL production screening for the most resistance *Klebsiella* spp.

Klebsiella spp.	Antibiotics			
	CAZ	CCA	CTX	CCT
40	0	18	0	17
47	0	18	0	18
49	15	26	13	28
62	0	10	0	9
82	0	19	0	17
90	12	19	0	8
92	17	26	14	23
103	0	17	0	19
104	9	17	7	18
105	12	22	15	23
107	11	19	21	29
108	10	23	15	23
112	12	21	15	27
113	16	26	23	31
114	0	17	0	19
K. pneumoniae (ATCC 700603)	0	18	0	16

CAZ: Ceftazidime 30µg; **CCA**: Ceftazidime/Clavulanic 30/10µg**; CTX**: Cefotaxime 30µg; **CCT**: Cefotaxime/Clavulanic acid 30/10µg.

Table (3), demonstrates the highest resistance fifteen ESBL *Klebsiella* spp. as determined by MIC which displayed a greater conc. than 1024 µg/ml against cefotaxime. However, they exhibited varying degrees of resistance to ceftriaxone and cefotaxime, respectively.

Table 3: Minimum inhibitory concentration profile of ESBL *Klebsiella* spp.

Klebsiella spp.	MIC (µg/ml)			Interpretation final result
	CRO	CTX	CAZ	
40	>1024	>1024	>1024	Resistant
47	512	>1024	>1024	Resistant
49	>1024	>1024	1024	Resistant
62	>1024	>1024	>1024	Resistant
82	>1024	>1024	>1024	Resistant
90	>1024	>1024	>1024	Resistant
92	>1024	>1024	128	Resistant
103	>1024	>1024	>1024	Resistant
104	>1024	>1024	128	Resistant
105	>1024	>1024	>1024	Resistant
107	>1024	>1024	>1024	Resistant
108	>1024	>1024	>1024	Resistant
112	>1024	>1024	>1024	Resistant
113	>1024	>1024	>1024	Resistant
114	>1024	>1024	256	Resistant
***E. coli* ATTC 25922**	1	1	4	Susceptible

MIC: Minimum inhibitory concentration**; CRO**: Ceftriaxone 30µg; **CTX**: Cefotaxime 30µg; **CAZ**: Ceftazidime 30µg

Table (4) illustrates the MICs of the highest fifteen resistance ESBL- *Klebsiella* spp. among different antibiotic classes including fluoroquinolone, aminoglycosides, phenicols and carbapenems. The results showed that imipenem, chloramphenicol and ciprofloxacin antibiotics revealed a diverse in sensitivity action among the tested isolates. While amikacin and ciprofloxacin as their breakpoint in resistant case must be ≥64 and ≥4 correspondingly, showed a high incidence of resistant MIC among tested species.

Table 4: Minimum inhibitory concentration of ESBL *Klebsiella* spp. among different antibiotic classes.

Klebsiella spp.	Ak		C		CIP		IMP	
	MIC µg/ml	Result	MIC µg/ml	Result	MIC µg/ml	Result	MIC µg/ml	Result
40	>1024	R	16	R	256	R	1	S
47	>1024	R	128	R	64	R	1	S
49	>1024	R	16	I	128	R	1	S
62	512	R	16	I	64	R	16	R
82	>1024	R	16	I	32	R	64	R
90	>1024	R	128	R	128	R	4	R
92	512	R	32	R	128	R	1	S
103	256	R	32	R	64	R	1	S
104	>1024	R	16	I	64	R	1	S
105	>1024	R	32	R	256	R	32	R
107	512	R	16	I	64	R	1	S
108	128	R	16	I	32	R	8	R
112	>1024	R	16	I	128	R	8	R
113	256	R	16	I	64	R	1	S
114	>1024	R	16	I	128	R	8	R
***E. coli* ATTC 25922**	32	I	8	S	0.5	S	1	S

S: Susceptible, **I**: Intermediate, **R**: Resistant **AK**: Amikacin 30µg; **C**: Chloramphenicol 30µg; **CIP**: Ciprofloxacin 5µg; **IMP**: Imipenem 10µg.

DISCUSSION

Klebsiella pneumoniae, one of clinical isolates which are considered as a causative agent of hospital acquired infection. Since 2015, the World Health Organization has documented an increase in *Enterobacteriaceae* resistance to third-generation cephalosporins, with *E. coli* and *K. pneumoniae* demonstrating the highest levels of resistance (WHO, 2021). The systematic study by Nasser and his colleagues emphasizes the variations in antibiotic resistance patterns between *Escherichia coli* and ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) in different countries in the Arabic region infections in various Arab nations, underscoring the difficulty in choosing the right antimicrobials for each patient's unique course of therapy.[15]

Our study identified 115 *Klebsiella* spp. out of a total of 181 isolates. The results declared that the highest prevalence species was *K. pneumoniae* subsp. pneumoniae representing 89% followed by *K. pneumoniae* subsp. *ozaenae* and *K. oxytoca* revealing 7 and 4% out of 115 total isolates. *Klebsiella* are nonmotile, facultative anaerobic rods. Most *Klebsiella* spp. are encapsulated, with some having a mucoid appearance. *K. pneumoniae* and *K. oxytoca* are distinguished by indole production, while *K. ornitholytica* is also an indole producer.[16] Wu and colleague concluded that *K. ozaenae* need to be taken just as seriously as other dangerous Gram-negative bacteria. However, under specific conditions, it can cause deaths in humans as well as severe invasive infections.[17]

Our results, which show that of 115 *Klebsiella* species, *K. ozaenae* displayed 6% identified confirmed using VITEK2, are almost identical with Radji and colleagues' cross-septentrional retrospective study, which stated that *K. ozaenae* represented 8% isolates out of total 249 positive cultures.[18] In comparison with our results only 53 (46%) out of 115 *Klebsiella* spp. were ESBL-producing *Klebsiella* spp. Garza-Ramos and colleague[19] have mentioned that the cephalosporin-resistant isolates cefotaxime and ceftazidime or both were identified in 38.4% of the samples and displayed a resistance of 78 and 74%, respectively. Also, they mentioned that ESBL producers were identified in 10.2% of the isolates that was in contrary of our results as ESBL producers were identified in 46% of the isolates. As well as our results revealed that susceptibility profile of these ESBL-producing isolates was 100% resistant to ampicillin and ceftazidime which agree with Garza-Ramos and his colleague[19] but in contrary with their results in 50% resistant to ciprofloxacin 100% susceptible to amikacin.

A study by Farhadi et al.[20] declared that the frequency of multidrug-resistant (MDR) and extensively drug-resistant (XDR) isolates in hospitals were 58 and 13%, respectively. Also, the highest and lowest resistance rates were related to ampicillin/sulbactam (93%) and amikacin (8%), respectively. In comparison with our data, it has been reported that 7.5, 16.1, 32.9, 34.1, 36.4, and 42.7% of the clinical *K. pneumoniae* isolates were resistant to imipenem, ciprofloxacin, trimethoprim-sulfamethoxazole, cefepime, amikacin, and ceftazidime, respectively.[21] Consequently, their findings are significantly higher than ours and those of Farhadi et al., who discovered variations in their investigation as a consequence of factors such sample size, research date, geographical distance, degree of sanitation, specimen type, and limitations on the use of antibiotics. It was suggested that the overuse of antibiotics could increase the drug-resistant bacteria and leads to the emergence of XDR *K. pneumoniae*.[22] A recent study revealed that 73.1% of *K. pneumonia* are resistant to at least one antibiotic.[23] Also, Miftode *et al*.[24] found that extreme use of β-lactam antibiotics has increased the prevalence of ESBL-producing *K. pneumonia*, contributing to nearly 45% of nosocomial infections and resulting in higher mortality rates.

According to a study conducted in 2023 by Attia and others,[25] *K. pneumoniae* is a highly common infection that affects children and newborn wards in Egypt. Among the investigated isolates, a high level of XDR (47.8%) followed by MDR (41.3%) resistance was found. These were almost agreed with our results *K. pneumoniae* represented 89% out of total isolates with XDR (45%) while, MDR and pan drug-resistant (PDR) represented (14 and 10%), respectively. Comparable to our findings a significant incidence of *K. pneumoniae* (73%, 51.35%) was recorded in some Egyptian research.[26,27] Also, Hassuna et al.[28] reported that among isolated *K. pneumoniae* 83.3% were XDR, while the rest were MDR isolates. In contrary of our results Nirwati et al.[29] recorded *K. pneumoniae* in 17.36% of all clinical bacterial isolates. Like our study, a high level of resistance to cephalosporins was reported.[27,30,31] Likewise, our findings are in concordance with previous reports.[25,28,30,32]

CONCLUSION

In our study, *K. pneumoniae* subsp. *pneumoniae* was the most represented *Klebsiella* species; however, *K. pneumoniae* subsp. *ozaenae*, and the *K. oxytoca* were considered as a cause of community-acquired infections. Highest resistance was identified among ESBL-producing clinical isolates. Also, our results indicate extended drug resistance among non–ESBL-producing isolates. In conclusion, diverse *Klebsiella* species with varying antibiotic resistance profiles are an indicator of the community acquired infections.

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