WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 2, 192-198. Research Article ISSN 2277– 7105

DETECTION OF MULTI DRUG RESISTANT AND EXTENDED SPECTRUM ß-LACTAMASES PRODUCING KLEBSIELLA SPECIES IN KHARTOUM STATE, SUDAN - 2019

Sahar Mohammed Seed Ahmed*[1] and Hassan Abd Alaziz Musa[2]

[1]Faculty of Medical Laboratory Sciences Omdurman Ahlia University.

[2]Faculty of Medicine The National Ribat University.

Article Received on
14 Dec. 2019,

Revised on 03 Jan. 2020,
Accepted on 24 Jan. 2020,

DOI: 10.20959/wjpr20202-16746

***Corresponding Author**
Dr. Sahar Mohammed Seed Ahmed
Faculty of Medical Laboratory Sciences Omdurman Ahlia University.

ABSTRACT

Klebsiella species; are commensal of the gastrointestinal tracts of human and animal. *Klebsiella pneumoniae* is the commonest human pathogen, known to be resistant to the beta lactam agents, by the production of Extended Spectrum Beta Lactamases (ESBLs). This study was conducted to detect the Multi drug resistant and ESBLs *Klebsiella* spp in Sudanese patients using phenotypic method. A total number of 300 *Klebsiella* spp were included. Antibiotic sensitivity was done, 79 of isolates showed multi drug resistance. The multi drug resistant strain were further identified using API 20 E system. Twenty five out of the 79 MDR strains were identified as *K. pneumoniae* (22) and *K. oxytoca* (3). Phenotypic detection for ESBL was positive result for the 25 isolates. The antibiotic resistance in the 25 isolate was very high for most of the used antibiotics. They were sensitive to colistin (96%) and imipenem (92%). and all MDR *Klebsiella* spp. As conclusion the commonest isolates of MDR and ESBLs producer in Sudan was *Klebsiella pneumonia*e & *Klebsiella oxytoca*. They were resistant to most of the used antibiotics except imipenem and colistin.

KEYWORDS: Extended Spectrum ß- Lactamase (ESBL), Multi Drug Resistant (MDR), Analytical Profile Index(API).

INTRODUCTION

Klebsiella species; are Gram-negative bacilli, belonging to the tribe *Klebsielleae* in the family *Enterobacteriaceae*.[1] *Klebsiellae* spp are normally present in the environment, and mucosal surfaces of humans and animals.[2]

They are considered as a common pathogens of urinary tract infections, wound infection, and neonatal septicaemia. They can be isolated from many unusual infections including endocarditis, peritonitis, muscle abscesses, and arthritis. They are opportunistic pathogens among immunocompromised patients.[3]

Klebsiella pneumoniae, is one of the most causative agents of hospital acquired infections which exhibits multi drugs resistance (MDR).[4]

They have antibiotics –resistant plasmids, that control the production of ß-lactamases which provide resistance against multiple antibiotics including beta lactam agents like amoxicillin, ticarcillin, third generation cephalosporins as cefotaxime, ceftriaxone, cefpodoxime and ceftazidime.[5]

ESBLs of *Klebsiella* spp were discovered early in 1980, and capable of hydrolyzing pencillins and broad spectrum cephalosporins. ESBLs are derived from TEM and SHV–type enzymes. They are transferable from strain to strain and between bacteria species.[5, 6,7]

A descriptive study carried out in Khartoum state hospitals during the period of June, 2007 to April, 2008. aiming to evaluate emergence of ESBL among multi drug resistant *E. coli* and *Klebsiella* species causing nosocomial UTI. *E. coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were among the studied isolates (49, 38 and 13% respectively). β-Lactamase was produced by all isolates; high resistance level for 3rd generation cephalosporin was noticed.[8] Another study done in New York City. 19 isolates of *Klebsiella* species producing carbapenem-hydrolyzing β-lactamase were recovered from November 1997 to July 2002 from 7 different hospitals. Most isolates were resistant to aminoglycosides and fluoroquinolones**.** During a study period, the prevalence of MDR among ESBL isolates increased from 12.5% to 26.9%.[9]

This study was done for the detection of Multi Drug resistant and phenotypic detection of ESBLs producing *Klebsiella* spp among Sudanese patients.

MATERIAL AND METHODS

This is a cross sectional laboratory based study. Ethical clearance was obtained from the National Ribat committee and from the Hospitals. Three hundred Isolates of *Klebsiella* spp were collected from different hospitals in Khartoum state. The specimens were cultured on MAC agar and blood agar media and incubated aerobically at 37°C overnight. All colonies were examined by gram stain, biochemical tests using, API 20E system. Antimicrobial sensitivity testing was done according to the Kirby and Bauer method. The antibiotics used were co-trimoxazole, cephalexin, ciprofloxacin, ceftriaxone, ceftazidime, cefuroxime, gentamicin, colistin and imipenem. Detection of the extended spectrum ß-lactamase was done phenotypicallly using the double disc diffusion method.

RESULTS

Out of the 300 isolates of *Klebsiella* spp identified biochemically 79 were Multi drug resistant (MDR) and 221 were non MDR. The result of antibiotics sensitivity found that resistance to Cephalexin represent 74(93.6%), Cefuroxime 50(63.2%), Ceftazidime 61(77.2%), Ciprofloxacin 69(87.3%), Co-trimaxazole 62(78.4%), Ceftriaxone 61(77.2%), Gentamicin 63(79.7%), Colistin 1(1.3%) and Imipenem 16(20.2%). (Table 1)

By using API 20 E system 25 of the multi drug resistant Klebsiellae isolates were *Klebsiella pneumonia*e and *Klebsiella oxytoca*. The remaining isolates were other *Klebsiella* spp.

Phenotypic method for the ESBLs was done for the 25 of *Klebsiella* spp isolates to detect ESBLs production. All isolates gave positive result in ESBLs phenotypic detection. (Figure1) The result of antibiotics sensitivity for the ESBLs producing isolates found that Cephalexin represent (96%), Cefuroxime (100%), Ceftazidime (100%), Ciprofloxacin (88%), Co-trimaxazole (88%), Ceftriaxone (92%), Gentamicin (76%), Colistin (4%) and Imipenem (8%). (Table 2)

Table 1: Percentage of the multi drug resistant (MDR) isolates by using different types of antibiotics.

Name of antibiotic	number sensitive	number resistant
Cephalexin	5(6.3%)	74(93.6%)
Cefuroxime	29(36.7%)	50(63.2%)
Ceftazidime	18(22.7%)	61(77.2%)
Ciprofloxacin	10(12.7%)	69(87.3%)
Co-trimaxazole	17(21.5%)	62(78.4%)
Ceftriaxone	18(22.8%)	61(77.2%)

Getamicin	16(20.2%)	63(79.7%)
Colistin	78 (98.7%)	1(1.3%)
Imipenem	63(79.7%)	16(20.2%)

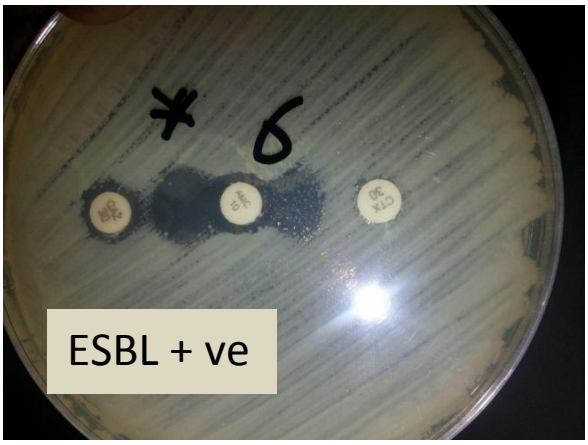

Figure 1: Phenotypic detection of ESBL, showing key whole indicating positive result.

Table 2: The antibiotics sensitivity of the 25 *Klebsiella pneumonia*e and *Klebsiella oxytoca.*

Name of antibiotic	number sensitive	number resistant
Cephalexin	1(4%)	24(96%)
Cefuroxime	0(0%)	25(100%)
Ceftazidime	0(0%)	25(100%)
Ciprofloxacin	3(12%)	22(88%)
Co-trimaxazole	3(12%)	22(88%)
Ceftriaxone	2(8%)	23(92%)
Getamicin	6(24%)	19(76%)
Colistin	24(96%)	1(4%)
Imipenem	23(92%)	2(8%)

DISCUSSION

Antimicrobial sensitivity testing was done in this study for 300 isolates of *Klebsiella* spp by using different types of antibiotic disks. The results obtained were 79 (26.3%) of isolates were Multi Drug Resistant (MDR) and 221(73.6%) of the isolates were non Multi Drug Resistant. This result agrees with the study done by Alves *et al* in Brazil and Hyle *et al* in philadelphia who reported that 68 of *Klebsiella* spp out of 361 isolates were MDR.[10,11] It disagrees with the study done in Kurdistan by Salimizand *et al* who reported that 46 of the total isolate were MDR and 21 were non MDR.[12]

The isolates of Multi Drug Resistant *Klebsiella* spp were tested using API 20 E system, to idetify the different species 25 of the isolates were identified as *Klebsiella pneumoniae* (22)

and *Klebsiella oxytoca* (3). This result was consistent with Holmes *et al* study in London using API 20 E system.[13]

Extended spectrum β-lactamases (ESBLs) are enzymes that resist the β-lactams agents. Phenotypic test was done for the *Klebsiella* isolates to detect ESBLs production. 25 of the isolates gave positive result in ESBLs production by the phenotypic detection method. A study done in France and England by Podschun and Ullmann reported similar results. However the result was different to a study done by Podschun and Ullmann in the united state.[14]

The result of antibiotic resistance match with the study done in india by Babypadmini and Appalaraju in the percentage of resistance to the third generation cephalosporins[15] and differ from another study done in china by Yu and *et al* that reported 60% were sensitive to third generation cephalosporins.[16]

CONCLUSION

The antibiotics that score high percentage of resistance among the total *Klebsiella* isolates were Cephalexin and Ciprofloxacin.

The commonest MDR and ESBLs producing isolates were *Klebsiella pneumonia*e and *Klebsiella oxytoca*.

ACKNOWLEDGEMENTS

We would like to acknowledge the staff at the Departments of Microbiology Laboratories Hospitals: in Soba University Hospital, Ribat Hospital, Omdurman Pediatric Teaching Hospital, Omdurman Teaching Hospital and Bahri Hospital for their participation in collection of the specimens.

REFERENCES

1. Srivastava S. Understanding bacteria. Springer Science & Business Media, 2003 Nov 30.
2. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical microbiology reviews, 1998 Oct 1; 11(4): 589-603.
3. Du J, Li P, Liu H, Lü D, Liang H, Dou Y. Phenotypic and molecular characterization of multidrug resistant Klebsiella pneumoniae isolated from a university teaching hospital, China. PloS one, 2014 Apr 16; 9(4): e95181.

4. Salimizand H, Shahcheraghi F, Kalantar E, Badmasti F. Molecular characterization of class 1 integrons and gene cassettes in multidrug resistant (MDR) Klebsiella spp. isolated from hospitalized and outpatients in Iran, 2009. Iranian journal of microbiology, 2013 Mar; 5(1): 48.
5. Manenzhe, G.I., *Detection and characterization of ESBL positive Klebsiella pneumoniae clinical isolates* (Doctoral dissertation, University of Pretoria), 2012.
6. Gupta A, Ampofo K, Rubenstein D, Saiman L. Extended spectrum β lactamase-producing Klebsiella pneumoniae infections: a review of the literature. Journal of perinatology, 2003 Sep; 23(6): 439.
7. Pessoa-Silva CL, Moreira BM, Almeida VC, Flannery B, Lins MA, Sampaio JM, Teixeira LM, Miranda LV, Riley LW, Gerberding JL. Extended-spectrum β-lactamase-producing Klebsiella pneumoniae in a neonatal intensive care unit: risk factors for infection and colonization. Journal of Hospital Infection, 2003 Mar 1; 53(3): 198-206.
8. Mekki AH, Hassan AN, Elsayed DE. Extended spectrum beta lactamases among multi drug resistant Escherichia coli and Klebsiella species causing urinary tract infections in Khartoum. African Journal of Bacteriology Research, 2010 Aug 23; 2(3): 18-21.
9. 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 TEM-30 β-lactamases in New York City. Clinical infectious diseases, 2004 Jul 1; 39(1): 55-60.
10. Alves MS, da Silva Dias RC, de Castro AC, Riley LW, Moreira BM. Identification of clinical isolates of indole-positive and indole-negative Klebsiella spp. Journal of clinical microbiology, 2006 Oct 1; 44(10): 3640-6.
11. Hyle EP, Lipworth AD, Zaoutis TE, Nachamkin I, Fishman NO, Bilker WB, Mao X, Lautenbach E. Risk factors for increasing multidrug resistance among extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella species. Clinical infectious diseases, 2005 May 1; 40(9): 1317-24.
12. Salimizand H, Shahcheraghi F, Kalantar E, Badmasti F. Molecular characterization of class 1 integrons and gene cassettes in multidrug resistant (MDR) Klebsiella spp. isolated from hospitalized and outpatients in Iran, 2009. Iranian journal of microbiology, 2013 Mar; 5(1): 48.
13. Holmes B, Willcox WR, Lapage SP. Identification of Enterobacteriaceae by the API 20E system. Journal of clinical pathology, 1978 Jan 1; 31(1): 22-30.

14. Podschun R, Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical microbiology reviews, 1998 Oct 1; 11(4): 589-603.
15. Babypadmini S, Appalaraju B. Extended spectrum-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae-prevalence and susceptibility pattern in a tertiary care hospital. Indian Journal of Medical Microbiology, 2004 Jul 1; 22(3): 172.
16. Yu Y, Ji S, Chen Y, Zhou W, Wei Z, Li L, Ma Y. Resistance of strains producing extended-spectrum β-lactamases and genotype distribution in China. Journal of Infection, 2007 Jan 1; 54(1): 53-7.