

Identification and Antibiotic Susceptibility Testing of ESBL Producing Klebsiella Strains by Phenotypic Methods

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ABSTRACT

Multi drug resistance has now become a worldwide therapeutic challenge due to the widespread use of broad spectrum antibiotics. Klebsiella species have significant importance in clinical field as they cause various infections in human and are considered as potential pathogens that express antibiotic resistance through their strong enzymatic activity. Extended spectrum beta lactamases (ESBLs) are plasmid mediated enzymes produced mostly because of mutation and few other factors. These enzymes confer resistance against various β -lactam drugs including cephalosporins and monobactams. Among the genus Klebsiella, ESBLs are highly prevalent in *K. pneumoniae* followed by *K. oxytoca*. This study was conducted in Pakistan to assess the distribution of ESBL producers among Klebsiella spp., an important member of the family Enterobacteriaceae. From January 2010 to January 2012, a total of 236 gram-negative isolates were collected from different renowned microbiological laboratories. Out of the 236 gram-negative isolates, 125 were found as Klebsiella spp. by using standard microbiological techniques. Antimicrobial susceptibility profiling of these strains was performed by using Kirby Bauer disk diffusion method. Phenotypic detection of the production of extended spectrum beta lactamase enzyme was performed using double disc synergy method and combination disc method. It has been identified that Klebsiella strains are highly resistant against Amoxicillin, Tetracycline, Nalidixic Acid, Cephadrine, Gentamicin, co-amoxyclov with the percentage of 100%, 86%, 86%, 82%, 82% and 80% respectively. The most effective antibiotics for Klebsiella spp. were found to be Amikacin, Meropenem and Piperacillin-tazobactam, with highest sensitivities of 96%, 94% and 91%. Phenotypic detection of Extended spectrum beta lactamase production by double disc synergy test was able to identify 28% ESBL producers among Klebsiella isolates whereas 64% were detected by combination disc test.

Keywords:

ESBL, Klebsiella, DDST, CDT.

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INTRODUCTION

Klebsiella is a well-known genus of the family Enterobacteriaceae associated with nosocomial and community-acquired pneumonia with high morbidity and mortality rates, if not treated timely and properly. Klebsiella as an opportunistic pathogen is mainly involved

in nosocomial infections affecting immunocompromised individuals more frequently¹. *Klebsiella pneumoniae* followed by *Klebsiella oxytoca* are the most important species of the genus Klebsiella, found to be associated

with human clinical specimens, accounting for 75% to 86% and 13% to 25% isolates respectively².

The continuous spread of drug resistance among pathogenic microbes has become a therapeutic challenge all over the world. The indiscriminate and widespread use of extended spectrum antibiotics has played a significant role for the spread of multiple drug resistance among pathogenic strains³. Klebsiella isolates are gaining importance due to the emergence of their multidrug resistant strains, specifically extended-spectrum beta lactamase(ESBL)-producing strains, capable of hydrolyzing beta lactam drugs¹. The first strain capable of producing ESBL was detected in Germany in 1983 and later several other outbreaks were reported in USA and other regions due to these ESBL producers⁴.

ESBLs are plasmid mediated enzymes that are capable of transferring resistance from one strain to another, not only for beta lactam drugs, they may also carry resistance genes of aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfamethoxazole-trimethoprim. These ESBLs can hydrolyze third generation cephalosporins including cefotaxime, ceftriaxone, ceftazidime and monobactams but could be inactivated by serine β -lactamase inhibitors such as clavulanate, sulbactam and tazobactam when used in combination with third generation cephalosporins³.

The timely and accurate detection of ESBL producers can prevent several outbreaks and endemics. Although various molecular techniques are now known for the detection of ESBL producers, however, in routine lab practice, they are rarely followed. It is therefore suggested to use two or more phenotypic detection methods for identification of ESBL producers in routine clinical settings to avoid misuse of antibiotics³. Prevalence, susceptibility profile and phenotypic characteristics of ESBL producers may vary from country to country and region to region. Therefore, in our study, we performed susceptibility testing and phenotypic detection of ESBL producer and ESBL non-producers among Klebsiella strains to establish effective antibiotic strategy and suggest appropriate empirical therapy in high-risk units.

The present study was carried out to determine the antibiotic resistance pattern and phenotypic and detection of resistance enzymes in Klebsiella species for

implementing the strategies and judicious use of drugs for proper therapy. For executing strict infection control policy and to stop the spread of resistant strains, it is important to detect ESBL producing isolates more promptly.

MATERIALS AND METHODS

Bacterial Isolates:

From January 2010 to January 2012, a total of 236 gram-negative isolates were collected from renowned microbiological laboratories of Karachi, Pakistan. All the samples were aseptically streaked on Nutrient Agar slants (Oxoid). Identification and characterization of bacterial isolates was performed by using standard microbiological techniques⁷. Identified isolates were stored at -70°C in Glycerol stock⁸.

Antibiotic Susceptibility Testing:

Antibiotic Susceptibility testing was performed by Kirby-Bauer method. Antimicrobial susceptibilities of the isolates to 25 different antimicrobial agents (μ g) viz. Amikacin (30), Gentamicin (10), Tobramycin (10), Amoxicillin (20), Amoxicillin/clavulanic acid (20/10), Piperacillin-tazobactam (100/10), Ciprofloxacin (5), Ofloxacin (5), Enoxacin (10), Sparfloxacin (5), Nalidixic Acid (30), Cephadrine/ Velosef (30), Cefuroxime (30), Cefixime (5), Cefotaxime (30), Ceftazidime (30), Ceftriaxone (30), Cefoperazone/Sulbactam (75/30), Imipenem (10), Meropenem (10), Doxycycline (30), Tetracycline (30), Trimethoprim/sulphamethoxazole (1.25/23.75), Fosfomycin(200) and Nitrofurantoin (300) were determined by using commercially available disks (Oxoid) and results were interpreted as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines⁹.

Phenotypic Detection of ESBL Producers:

Extended spectrum beta lactamase producing strains among Klebsiella isolates were detected by the methods of double disc synergy and combination disc.

I. Double Disc Synergy Test:

The test was performed by using disks of Cefotaxime (30 μ g) (Oxoid™) and Amoxicillin Clavulanic acid (20/10 μ g) (Oxoid™). Disc of Cefotaxime was placed at 15mm from Amoxicillin Clavulanic acid disc and incubated at 37°C for 24 hours. Synergy between the discs was observed and the increased zone of CTX towards AMC was

considered as a clear indication for ESBL production¹⁰.

II. Combination Disc Method:

Cefotaxime (30ug) (Oxoid™) and the combined disc of Cefotaxime plus Clavulanic acid (30/10 ug) (Bioanalyse®) were used in this test following protocol recommended by CLSI in 2010. Both discs were placed at a distance of 24mm to observe the zones of inhibition. ESBL producers were detected by comparing the zone sizes of the discs. ESBL production can be interpreted by 5 mm increase in the zone size of combination disc as compared to the cefotaxime disc⁹⁻¹¹.

(86%), Gentamicin & Cephradine (82%) and Amoxicillin/clavulanic acid (Co-Amoxycrav) (80%).

Resistance pattern of Klebsiella strains against other antimicrobials was detected as 63% against Cefuroxime, 42% to Cefixime; 36% to Ciprofloxacin, 33% to Sparfloxacin, 30% to Enoxacin, 29% to Ofloxacin and Cefotaxime whereas 26% resistance was observed against Ceftazidime, Ceftriaxone and Cefoperazone/Sulbactam and 19% isolates were resistant to Tobramycin. Complete Resistance profile including number of sensitive, intermediate and resistant strains are mentioned in Fig. 2.

RESULTS

Out of 125 identified Klebsiella strains, 100 strains were isolated from female patients and 25 strains were from male patients. Klebsiella isolates were detected higher in the patients of younger age followed by the older group. Less number of isolates were detected in the middle-aged patients and children. Distribution of Klebsiella isolates with respect to the age group has been shown in Fig. 1.

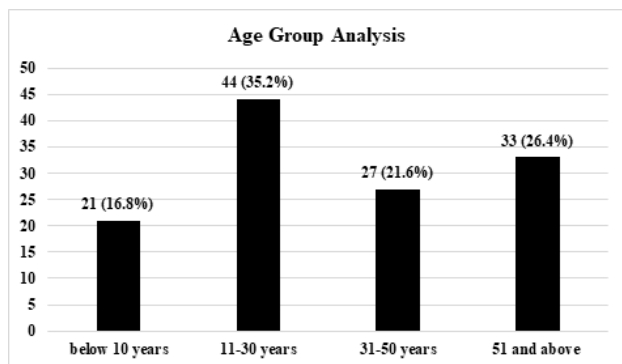


Fig. 1: Age group analysis showing the number of Klebsiella isolates collected from selected age groups.

Susceptibility testing of strains detected that amikacin, meropenem, imipenem and piperacillin-tazobactam are the most effective antimicrobial agents against Klebsiella isolates. It was found that only 8% isolates were resistant to Imipenem, 6% showed resistance against meropenem, 3% to Amikacin, and 2% to Piperacillin-tazobactam. We detected resistance against amoxicillin in 100% strains of Klebsiella as the isolates were intrinsically resistant to penicillin. Highest resistance was observed against Nalidixic acid (86%), Doxycycline (86%), Tetracycline

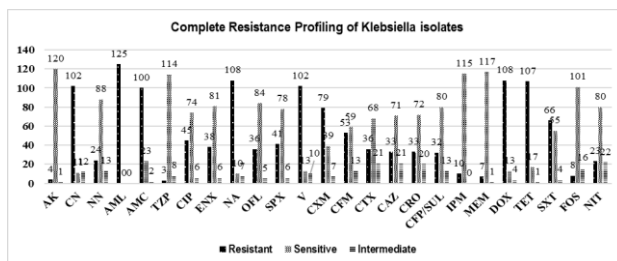


Fig. 2: Antibiotic resistance pattern of Klebsiella species against multiple drugs.

Out of 125 identified strains of Klebsiella, 35 were found as ESBL producers by Double disc synergy test whereas 80 strains were detected as ESBL producers by combination disc method as mentioned in Table 1. Detection of ESBL producers due to size and variation in zones of inhibitions for both methods has been illustrated in Fig. 3.

Table 1: Number and percentage of ESBL positive Klebsiella isolates using DDST and CDT.

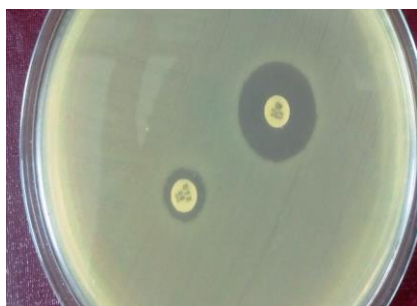
Test	No. of ESBL positive isolates(n) Percentage (%)
Double Disc Synergy Test	35 (28)
Combination Disc Test	80 (64)
Total No. of ESBL producers by both method	81 (64.8)

Double Disc Synergy Test



(A): Double Disc Synergy Test positive *Klebsiella* isolate showing keyhole appearance.

Combination Disc Test



(B): Combination Disc Test positive *Klebsiella* isolate showing extended zone of inhibition in CTX+CA as compared to CTX alone.

Fig. 3: Comparison of (A) Double Disc Synergy Test and (B) Combination Disc Test.

DISCUSSION

Several reports and studies have been conducted on *Klebsiella* isolates, describing their occurrence, susceptibility pattern and ESBL production. *Klebsiella* strains have gained immense importance among the scientific community due to their multi drug resistance strains with a specific mechanism of resistance i.e. enzyme production. In our study we identified 125 *Klebsiella* strains with high occurrence among females and less in males. Our findings confirmed the studies reported from India, Nigeria, Iran in which *Klebsiella* spp. were detected higher in females as compared to males due to the compressed size of urethra^{5,6,12-14}. We detected that the prevalence of *Klebsiella* isolates was higher in the younger age group. Contradictory researches were reported from India and Iran in which higher number of isolates were detected in the middle-aged group and the age group of children below 2 years respectively.

Dissimilarity in the result can be occurred in different regions, especially it depends upon the consumption rate of antibiotics among different age groups^{5,14}.

We determined the resistance profile of *Klebsiella* isolates by using twenty-five antimicrobial agents. We found that all *Klebsiella* isolates were found resistant to amoxicillin. Nalidixic Acid (NA), Doxycycline, Tetracycline, Gentamicin, Cephadrine, and Amoxicillin/clavulanate were less effective antimicrobials against *Klebsiella* isolates. Moderate resistance was observed against cephalosporins and quinolones. However, carbapenems, amikacin and Piperacillin-tazobactam have shown good inhibitory results against *Klebsiella* strains. All strains of *Klebsiella* were resistant against amoxicillin. Few other studies reported 100% resistance against amoxicillin however, comparatively less resistance against co-amoxiclav was reported in another research¹⁵. Moderate resistance to third generation cephalosporin and comparatively less resistance against piperacillin tazobactam and aminoglycosides¹⁶⁻¹⁸. Good susceptibility results were obtained with aminoglycosides and fluoroquinolones⁶.

In our study, we detected carbapenem as an effective drug against *Klebsiella* spp. However, contrary to our research, resistance against carbapenem drugs was also reported in *Klebsiella* isolates¹⁹. Although we did not obtain good inhibitory results from ciprofloxacin against *Klebsiella* isolates, however, few studies have reported ciprofloxacin as an effective drug against these strains^{16,20}. A study reported that carbapenem should be considered as a drug of choice against the infections caused by *Klebsiella* isolates²¹. A similar study conducted in Australia, revealed that imipenem has the similar susceptibility pattern against ESBL producing isolates and ESBL non-producing isolates²². *Klebsiella* strains were found to be sensitive against amikacin and imipenem in the study²³. Comparable results were reported in another study of Pakistan in which imipenem, piperacillin-tazobactam, ampicillin-sulbactam and amikacin were found as effective drugs²⁴.

We detected 35 (28%) and 80 (64%) strains of ESBL producers by Double disc synergy test and by combination disc method respectively. ESBL producers were initially reported from Europe in 1983, since then their prevalence is increasing day by day. Various reports

have revealed its prevalence in different regions of the world. ESBL production was detected in 30-60% isolates from Brazil in a study conducted on the patients of intensive care units²¹. In another study, 86% of *Klebsiella* strains were detected as ESBL producers by both methods¹⁷.

Combination Disc Method can be considered to be more reliable as it detects higher number of isolates in our research and this method was also verified by the fact that by adding clavulanic acid to third generation cephalosporin, it will increase its effectiveness. Similar phenomenon was stated in another research²⁰. Comparatively lesser number (24.5%) of ESBL producing strains were detected in Pakistan 2013²⁴. Another study from Pakistan reported 22% ESBL producing strains among *Klebsiella* isolates in 2016²⁵. In 2016, a study reported fewer number of ESBL producers by using the method of Double disc synergy as compared to the combination disc method²⁶. In a study conducted in Nepal, higher number of ESBL producing *Klebsiella* isolates (18.4%) were reported by using the method of phenotypic confirmatory disc diffusion test²⁷ whereas in 2017, ESBL production was reported in relatively less number of *Klebsiella pneumoniae* (3.8%) and *Klebsiella oxytoca* (0.7%) isolates²⁸. Therefore, it would be preferable to conduct more than phenotypic screening technique to detect the correct number of ESBL producers.

CONCLUSIONS

This is an alarming situation that antibiotic resistance is increasing among pathogenic strains. Therefore, now there is an intense need to restrict the widespread use or misuse of antibiotics. Antibiotic susceptibility profiling and detection of ESBL producing strains must be included in the routine laboratory testing protocols. In the light of our findings, we could suggest that the use of amoxicillin and co-amoxiclav should be restricted. Cephalosporins could only be used in case of ESBL non-producers. However, Carbapenems, Amikacin and Piperacillin-tazobactam can be the drugs of choice for the treatment of infections caused by *Klebsiella* species. Phenotypic screening of ESBL producers can be better detected by the combination disc test or it is preferable to use more than

one screening procedures for the detection of ESBL production.

REFERENCES

1. Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev.* 1998; 11(4):589-603.
2. Pokra M, Sharma DK, Mehta P, Verma HR, Pundir S, Rana J, *et al.* Its Alarming, *Klebsiella* spp. towards Multidrug Resistance. *Int J Curr Microbiol App Sci* 2016; 5(6): 150-60.
3. Vinodhini R, Moorthy K, Palanivel P, Punitha T, Saranya S, Bhuvaneshwari M, *et al.* Detection and antimicrobial susceptibility pattern of ESBL producing Gram negative bacteria. *Asian J Pharm Clin Res.* 2014;7(1):243-7.
4. Behrooz A, Rahbar M, Yousefi J.V. Frequency of extended spectrum beta-lactamase (ESBLs) producing *Escherichia coli* and *klebsiella pneumoniae* isolated from urine in an Iranian 1000-bed tertiary care hospital. *Afr J Microbiol Res* 2010; 4 (9): 881-8.
5. Akram M, Shahid M, Khan A.U. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC hospital Aligarh, India. *Ann Clin Microbiol Antimicrob* 2007; 23; 6: 4.
6. Chikwendu CI, Amadi ES, Obi RK. Prevalence and antimicrobial resistance in *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates from non-clinical urine samples. *NY Sci J.* 2010; 3(11): 194-200.
7. Krieg NR, Holt JC. (eds., 1984). *Bergey's Manual of Systematic Bacteriology*, 1st ed., vol. 1, Williams and Wilkins, Baltimore.
8. Cheesbrough M. 2006. A practical and well-illustrated guide to microbiological, hematological, and blood transfusion techniques. *District Laboratory Practice in Tropical Countries - Part 2.*
9. National Committee for Clinical Laboratory Standards (NCCLS) guidelines 2010.
10. Vaidya VK. Horizontal transfer of Antimicrobial Resistance by Extended-Spectrum β Lactamase-Producing Enterobacteriaceae. *J Lab Physicians* 2011; 3(1): 37-42.
11. Lin C-F, Hsu S-K, Chen C-H, Huang Jr-R, Lo H-H. Genotypic detection and molecular epidemiology of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a regional hospital in central Taiwan. *J Med Microb* 2010; 59: 665-71.
12. Khosravi AD, Hoveizavi H, Mehdinejad M. Prevalence of *Klebsiella pneumoniae* encoding genes for CTX-M-1, TEM-1 and SHV-1 extended-spectrum beta lactamases (ESBL) enzymes in clinical specimens. *Jundishapur J Microbiol* 2013; 6(10): e8256.

13. Babakhani S, Derikvand SS, Nazer MR, Kazemi MJ. Comparison frequency and determination antibiotic resistance pattern of *Klebsiella* spp. Isolated from nosocomial infection in Khorramabad shohadayeashayer hospital. *Bull Env Pharmacol Life Sci* 2014; 3(12): 149-54.
14. Akbar DA, Ali RA, Zahra KJ. Screening and prevalence of SHV/CTX-M/TEM β -lactamase resistance genes in *Klebsiella* strains isolated bacteria from urinary tract infection in pre-school-age children in Jahrom, Iran. *IIOABJ* 2016; 7 (10): 82-8.
15. Wagenlehner FME, Niemetz A, Dalhoff A, Naber KG. Spectrum and antibiotic resistance of uropathogens from hospitalized patients with urinary tract infections: 1994-2000. *Int J Antimicrob Agen* 2002; 19: 557-64.
16. Randrianirina F, Soares J-L, Carod J-F, Ratsima E, Thonnier V, Combe P, Grosjean P, Talarmin A. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in Antananarivo, Madagascar. *J Antimicrob Chemother* 2007; 59: 309-12.
17. Manchanda V, Singh NP, Goyal R, Kumar A, Thukral SS. Phenotypic characteristics of clinical isolates of *Klebsiella pneumoniae* & evaluation of available phenotypic techniques for detection of extended spectrum beta-lactamases. *Indian J Med Res* 2005;122: 330-7.
18. Kashaf N, Djavid GE, Shahbazi S. Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran, Iran. *J Infect Dev Ctries* 2010; 4(4):202-6.
19. Bratu S, Tolaney P, Karumudi U, Quale J, Mooty M, Nichani S, *et al.* Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, NY: molecular epidemiology and *in vitro* activity of polymyxin B and other agents. *J Antimicrob Chemother* 2005; 56: 128-32.
20. Colodner R. Extended-spectrum beta-lactamases: a challenge for clinical microbiologists and infection control specialists. *Am J Infect Control* 2005; 33(2):104-7.
21. Paterson DL, Bonomo RA. Extended spectrum β -lactamases: A clinical update. *Clin Microbiol Rev.* 2005; 18(4): 657-86.
22. Hawser SP, Bouchillon SK, Hoban DJ, Badal RE, Hsueh P-R, Paterson DL. Emergence of high levels of extended-spectrum-beta-lactamase-producing gram-negative bacilli in the Asia-Pacific Region: data from the study for monitoring antimicrobial resistance trends (SMART) Program, 2007. *Antimicrob Agen Chemother* 2009; p. 3280-4.
23. Abreu AG, Marques SR, Monteiro-Neto V, Gonçalves AG. Extended-spectrum beta-lactamase-producing enterobacteriaceae in community-acquired urinary tract infections in São Luís, Brazil. *Braz J Microb* 2013; 44(2): 469-71.
24. Ahmed I, Sajed M, Sultan A, Murtaza I, Yousaf S, Maqsood B, *et al.* The erratic antibiotic susceptibility patterns of bacterial pathogens causing urinary tract infections. *EXCLI J* 2015; 14: 916-25.
25. Ain NU, Abrar S, Nazeer K, Riaz S. Extended spectrum beta lactamases and metallo-beta-lactamases: An update from Lahore, Pakistan *Eur J Pharm Med Res* 2016; 3(9): 92-7.
26. Yala J-F, Mabika RM, Bisseye C, Kenguele HM, Kama EM, Dikoumba AC, *et al.* Phenotypic and genotypic characterization of extended-spectrum-beta-lactamases producing-Enterobacteriaceae (ESBLE) in patients attending Omar Bongo Ondimba military hospital at Libreville (Gabon). *J Mol Microbiol Biotechnol.* 2016; 4(6): 944-9.
27. Chaudhary P, Bhandari D, Thapa K, Thapa P, Shrestha D, Chaudhary HK, Shrestha A, Parajuli H, Gupta BP. Prevalence of Extended Spectrum Beta-Lactamase Producing *Klebsiella Pneumoniae* Isolated from Urinary Tract Infected Patients. *J Nepal Health Res Counc.* 2016;14(33): 111-5.
28. Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL Production Among *E. coli* and *Klebsiella* spp. Causing Urinary Tract Infection: A Hospital Based Study. *Open Microbiol J.* 2017; 11: 23-30.