Original Research Article

Drug resistance profile of Klebsiella Species from clinical isolates in a tertiary care hospital

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Abstract

Background: Klebsiella species are widely distributed in nature occurring as commensals in intestines and saprophytes in soil and water. These are associated with wide variety of opportunistic and nosocomial infections, such as pneumonia, U.T.I, wound infections and septicemia. Increasingly, they have developed antimicrobial resistance, notably to various Cephalosporins and Carbapenems resistance is also on the rise which is of growing concern worldwide. **Objectives:** Antibiotic susceptibility pattern of Klebsiella species along with E.S.B.L production from various clinical isolates. **Material and Methods:** A total of 244 isolates of Klebsiella species were obtained from various clinical samples identified by standard laboratory procedures. Isolates were subjected to susceptibility testing against various antibiotics by disc diffusion test as per C.L.S.I guidelines. Isolates resistant to 3rd Generation Cephalosporins were tested for E.S.B.L production by using phenotypic confirmatory tests. **Results:** Of the 244 isolates, highest isolation rate was from sputum (40.5%), followed by urine (38.5%), exudate (15.5%), blood (3.27%) and pleural fluid (2.04%). The resistance rate of Klebsiella species for 3rd Generation Cephalosporins was around (45%), Gentamicin (30.3%), Cotrimoxazole (33%), Ciprofloxacin (32%), Lomefloxacin (30%) and Levofloxacin (34%). Maximum sensitivity was seen against Piperacillin-Tazobactam (65%) and Imipenem (89.2%). 37% of isolates were confirmed by phenotypic confirmatory tests as E.S.B.L producers. **Conclusion:** Regular monitoring and judicious use of antibiotics helps in preserving the effectiveness of sensitive antibiotics and controls the emergence of further resistance.

Key Word: Klebsiella Species.

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INTRODUCTION

Klebsiella is ubiquitously present and these occur as commensals in intestines and saprophytes in soil and water. They are important pathogens in nosocomial infections and are leading causes of morbidity and mortality [1] and are associated with wide variety of opportunistic infections, such as pneumonia, U.T.I, wound infections and septicemia and have been well documented in India.[2] They exhibit Plasmid encoded resistance to broad spectrum Cephalosporins in GNBs which has become a widespread phenomenon in clinical medicine.[3] These antibiotics are inactivated by different ESBLs which have evolved by stepwise mutation of TEM/SHV type β- lactamases which result in high morbidity and mortality.[4]

MATERIAL AND METHODS

A total of 244 isolates of *Klebsiella* species were isolated from sputum, urine, blood, exudates and pleural fluid and were examined morphologically for colony characteristics on agar media and were processed and biochemical testing done. All isolates were identified as per the standard laboratory protocols.[5] The study was undertaken during September 2013 to August 2014. Isolates were subjected to susceptibility testing against various antibiotics by Kirby Bauer disc diffusion method as per C.L.S.I 2014 guidelines.[6] Positive control- *K. pneumoniae* ATCC 700603. Negative control- *E. coli* ATCC 25922

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RESULTS

The samples processed in the lab were sputum, urine, exudates, blood and pleural fluid. Out of the 244 samples, the organisms isolated were Klebsiella pneumoniae (200) and Klebsiella oxytoca (44). From the sputum sample, 84 (34.4%) of K. pneumoniae and 15 (6.1%) of K. oxytoca isolates were grown. 67 (27.4%) and 27 (11%) of K. pneumoniae and K. oxytoca isolates were grown in urine samples respectively. From exudates, there was a growth pattern of 35 (14.3%) and 3 (1.2%) of K. pneumoniae and K. oxytoca respectively. Only K. pneumoniae was isolated from blood 8 (3.27%) and pleural fluid 5 (2.04%).

Table 1: Phenotypic Methods- Screening test and Confirmatory Method

Test	Initial Screen Test	Phenotypic Confirmatory test
Test Method	Disk Diffusion	Disk Diffusion
Medium	Mueller Hinton Agar	Mueller Hinton Agar
Antimicrobial concentration	*K. pneumoniae*- Ceftriaxone-30 mcg Ceftazidime-30 mcg Aztreonam-30 mcg Cefotaxime-30 mcg	Ceftazidime-30 mcg Ceftazidime-Clavulanic acid-30/10 mcg (AND) Cefotaxime-30 mcg CefotaximeClavulanicAcid-30/10 mcg
Inoculum	Standard Disk Diffusion Procedure	Standard Disk Diffusion Procedure
Incubation	35+/-2 ^{o}C; ambient air	35+/-2 ^{o}C; ambient air

Table 2: Total number of isolates screened and confirmed for E.S.B.L production

Klebsiella species	Screened positive Ceftazidime (CAZ) Cefotaxime (CTX) Ceftriaxone (CTR)			Confirmed- (Ceftazidime Ceftazidime-Clavulanic acid)
	CAZ	CTX	CTR	
Klebsiella pneumonia (200)	60	84	56	54 27%
Klebsiella oxytoca (44)	7	6	8	5 10%

Figure 1

Figure 2

Legend

Figure1: Combined Disk method; **Figure 2:** Double Disk Synergy Test/Double Disk Diffusion confirmatory test

Table 3: Total Antibiotic Resistance Profile for Klebsiella species

Sl. No	Antibiotics	Klebsiella pneumoniae (200)	KlebsiellaOxytoca (44)	Total (244)
1	Levofloxacin -5 mcg	70 (35%)	32 (72%)	102 (41%)
2	Lomefloxacin-15 mcg	64 (32%)	32 (72%)	96 (39%)
3	Ciprofloxacin – 5 mcg	56 (28%)	28 (63%)	84 (34%)
4	Amikacin-30 mcg	66 (33%)	16 (36%)	82 (33%)
5	Amoxyclav-30 mcg	90 (45%)	32 (72%)	122 (50%)
6	Ceftriaxone -10 mcg	80 (40%)	24 (54%)	104 (43%)
7	Pipercillin-Tazobactam-100/10 mcg	50 (25%)	18 (40%)	68 (27%)
8	Gentamicin-10 mcg	58 (29%)	16 (36%)	74 (30%)
9	Imipenem-10 mcg	20 (10%)	15 (35%)	35 (14%)
10	Cefotaxime-30 mcg	106 (53%)	38 (86%)	144 (50%)
11	Cefoperazone-50 mcg	86 (43%)	25 (56%)	111 (45%)
12	Amoxicillin-10 mcg	90 (45%)	24 (54%)	114 (46%)

DISCUSSION

ESBL producing Klebsiella pneumoniae were extensively reported worldwide after it was first identified in Enterobacteriaceae isolates from India. In this study, the frequency of ESBL producing Klebsiella species was found to be 37%. Higher incidences of ESBL production (71.4%) amongst gram negative bacterial isolates have been reported by other workers.[7] Lower incidence of ESBL production (22%) was seen in Parul Agarwal *et al*study 2008.[8] The incidence of 40% and 36% ESBL production reported by Baby Padmini et al, 2008 and Ritu Agarwal et al, 2009 respectively was in concordance with the present study.[9, 10] Isolates from sputum samples were found to be maximum 99/244 (40.5%) and among them Resistance against Ceftriaxone-60%, Cefotaxime-59%, Amoxyclav-50%, Amoxicillin-46%, Cefoperazone-45%, Levofloxacin-34%, Lomefloxacin-30%, Ciprofloxacin-32%. E.S.B.L production was 15%. This could be due to injudicious use of antibiotics in patients suffering with recurrent respiratory tract infections and on ventilators in I.C.U's. However, 89.2% sensitivity to Imipenem was observed. This advocates the use of Carbapenem antibiotics as a therapeutic alternative in the wake of increasing resistance rates observed with conventional β-lactam antibiotics.[11] 67% sensitivity towards Amikacin was seen. Next to Carbapenems, Aminoglycosides can be alternative to the third generation Cephalosporins for the treatment of serious infections due to E.S.B.L producing gram negative bacteria. Outbreaks of health care-associated infection caused by K. oxytoca have most often been associated with contamination of environmental reservoirs such as disinfectants, multi-dose vials or parenteral fluid bags, humidifiers and ventilators.[12] This suggests that hand-washing sinks in high-intensity hospital care areas may be a reservoir for K. oxytoca and that person-to-person transmission may also occur.

CONCLUSION

Statistical data and evidences from researchers prove that multi drug resistant bacteria are emerging worldwide which causes many public health problems and challenges to health care. Use of broad spectrum antibiotics, insufficient aseptic conditions and technique with inadequate control of infections spread has aggravated this problem. We should take immediate action to strengthen surveillance and laboratory capacity. The physicians should also promote rational use of medicines to avoid antibiotic drug resistance.

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