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Occurrence of Extended Spectrum Beta Lactamase Producing *Klebsiella* Species in Clinical Samples

KEYWORDS: *Klebsiella* species, ESBL, Cephalosporins, PCDDT

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ABSTRACT *Klebsiella species are important and leading in causing nosocomial infections and associated risk factors and being hard to eradicate due to development of multidrug resistant strains that produce extended spectrum beta lactamase (ESBL) enzymes. The aim of this study was to find out the incidence of ESBL producing Klebsiella species from clinical samples. A total of 437 clinical isolates of Klebsiella isolated from 1081 clinical samples and amongst them K. pneumoniae was the commonest (385) followed by K. oxytoca (51), while K. ozanae had only one isolate. All isolates were examined for ESBL production according to CLSI guidelines. The result showed that, 100 isolates of Klebsiella comprising of 66 and 34 from Amravati and Akola districts, respectively were expressed ESBL enzymes. There was no significant difference in prevalence of ESBL producing Klebsiella isolated among various clinical samples collected from Amravati and Akola city. Tests for detection of ESBL producing bacteria should be carried out at all diagnostic centers routinely and use of third generation cephalosporins should be restricted. This can reduce the prevalence of ESBL producing organisms.*

INTRODUCTION

Klebsiella is Gram negative, non motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium (Sarathbabu et al., 2012). Klebsiella species is ubiquitously present and important pathogen in nosocomial infections reported worldwide (Sikarwar & Batra, 2011). Klebsiella associated with various infections such as UTI, Septicemia, Wound infections, respiratory tract infections and diarrhea (Shubha & Ananthan, 2002). Epidemic and endemic hospitalized infections caused by Klebsiella species are leading causes of morbidity and mortality (Cryz et al., 1985). The wide spread use of antibiotics in hospitals has led to emergence of multidrug organisms of low virulence like Klebsiella causing serious opportunistic infections (Palucha et al., 1999).

The resistant strains of Klebsiella gain their resistance by producing ESBL enzymes (Jennifer et al., 2005). ESBL is mostly chromosomal or plasmid encoded that catalyze the hydrolysis of beta- lactam C-N bond antibiotics to give beta amino acid devoid of antibacterial activity (Mascaretti., 2003). The genes responsible for horizontal transfer of antibiotic resistance can be present in chromosomes or plasmid with in integrons (Vaidyanathan et al., 2009). ESBL are more prevalent in Klebsiella species than any other enterobacterial species (Manchanda et al., 2005). ESBL producing Klebsiella species were first reported in 1983 from Germany and since then a steady increase of strains resistant to cephalosporins has been reported with high prevalence worldwide (Shukla et al., 2004). The prevalence rate recorded in India is 13% to 87% (Jain & Mondal, 2007 and Manchandas et al., 2005). The national committee for clinical laboratory standards recommended ESBLs screening method and confirmatory tests (NCCLS, 2000). However their use in microbiology laboratory has been neglected. Delay in the detection and reporting of ESBL production by Klebsiella is associated with prolonged hospital stay and increased morbidity, mortality and health care costs (Kollef, 2003).

The present study was conducted to find out the prevalence of ESBL producing Klebsiella species in clinical samples collected from Amravati and Akola districts of Maharashtra state, India. The information would be useful in establishing empiric therapy guidelines and to contribute data to larger more extensive surveillance programs.

MATERIALLS AND METHODS

Sample collection

A total of 1081 different clinical samples viz., urine (385), pus (214), sputum (160), tracheal secretion (91), blood (94), oral thrush (37), CSF (50) and miscellaneous (50) were collected from Amravati and Akola district's hospitals and private pathology laboratories. The samples were collected with universal safety precautions and transported to the laboratory without delay. Samples were obtained from both outpatients and from those admitted to hospitals.

Isolation and Identification

All samples were inoculated on Mac Conkey agar, UTI agar and Mac Conkey agar (Hi-media) modified according to sample. The routine standard operative procedures are followed in the laboratory in isolating and identifying the Klebsiella from the clinical samples. Klebsiella species were identified by typical mucoid, lactose fermenting colony, Gram staining morphology, motility test, catalas, oxidase, Arginine Dihydrolase, Malonate Utilization, Ornithine Decarboxylase, Lysine Decarboxylase tests, IMViC reaction, fermentation of sugars like glucose, lactose, sucrose, mannitol with production of acid and gas.

Inoculum preparation

The inoculum was prepared in nutrient broth (Hi-media) and the turbidity of the broth was made equivalent to a 0.5 McFarland standard. Sterile cotton swab was soaked in this suspension and used to make lawn culture on Muller Hinton (Hi-media) agar plates.

Phenotypic confirmatory disc diffusion test (PCDDT) for ESBL production

The combined disc method was used to confirm the presence of ESBL on all the isolates of Klebsiella species by placing a disc of ceftazidime (30 µg) alone and ceftazidime (30 µg) in combination with clavulanic acid on a Muller Hinton agar plates. The discs were placed at least 20 mm apart from each other. A difference of ≥ 5mm between the zone of diameter around ceftazidime plus clavulanic acid and ceftazidime was taken to be phenotypic confirmation of ESBL production (NCCLS, 2005, Paterson & Bonomo, 2005).

RESULTS

In the present work, total 437 clinical isolates of Klebsiella

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were isolated from 1081 different clinical specimens, collected from Amravati and Akola. The overall prevalence of Klebsiella species colonization was observed to be 40.42%. Majority of Klebsiella were isolated from urine (165) followed by burn wound swab (130), sputum (72), tracheal secretion (25), blood (22), oral thrush (12), miscellaneous (10) and CSF (1). But the rate of prevalence of colonization was high in burn wound swab (60.70%) followed by sputum (45%), urine (42.9%), blood (23.40%), oral thrush (32.4%), tracheal secretion (27.27%), miscellaneous (20%) and CSF (2%). Among 227 urine samples collected from Amravati, 63 samples were confirmed urinary tract infected samples which showed high prevalence rate of 87.30%, while suspected samples showed 35.36%. A total three different Klebsiella species were recovered from clinical specimens, amongst them K. pneumoniae was the commonest (385) followed by K. oxytoca (51), while K. ozanae had only one isolate and that to reported in urine sample. The ESBL were detected using PCDDT method in all clinical isolates of Klebsiella. Furthermore, the formation of ESBLs in these strains was also evaluated, 22.88% were ESBL producer while 77.12% of them were non ESBL producers (Fig.1).

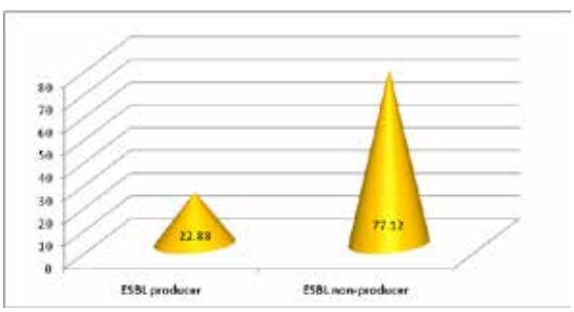

Fig 1: Rates of ESBLs producer and ESBL non producer in Klebsiella species.

Among total 100 ESBL positive Klebsiella strains, K. pneumoniae were 86%, while K. oxytoca 13% and K. ozanae 1%. ESBL production in different Klebsiella species is shown in table 1. Concerning ESBL production and the site of isolation, ESBL production was highest among Klebsiella isolated from blood (27.27%) followed by urine (24.24%), tracheal secretion (24%), sputum (19.44%), pus (18.46%), miscellaneous (18%), oral thrush (8.33%); while not a single ESBL positive Klebsiella isolated from CSF. In the present study, K. pneumoniae produced ESBL in a higher percentage than that of K. oxytoca. The total percentage of ESBL producing Klebsiella spp. isolated from Amravati and Akola districts were 21.93% and 20.25% respectively. Miscellaneous samples were not taken for comparison as specimen numbers of different samples were less in number. There was no significant difference in prevalence of ESBL producing Klebsiella spp. isolates among various clinical samples collected from Amravati and Akola districts as shown in table (2).

Table (1): ESBL production in different Klebsiella species

Specimen	Number of ESBL producing K. pneumoniae	Number of ESBL producing K. oxytoca	Number of ESBL producing K. Ozanae
Urine	33	06	01
Burn wound pus	23	01	--
Sputum	13	01	--
Tracheal secretion	03	03	--
Blood	06	--	--
Oral thrush	01	--	--
CSF	--	--	--
Miscellaneous	07	02	--
Total	86	13	01

Table (2): District wise distribution of ESBL producing Klebsiella species

Specimen	Number and % of ESBL producing Klebsiella from Amravati	Number and % of ESBL producing Klebsiella from Akola
Urine	29 (25.66%)	11 (21.15%)
Burn wound pus	13 (17.11%)	11 (20.37%)
Sputum	08 (20%)	06 (18.75%)
Tracheal secretion	04 (26.67%)	02 (20%)
Blood	04 (30.77%)	02 (22.22%)
Oral thrush	01 (8.33%)	--
CSF	--	--
Miscellaneous	07	02
Total	66	34

DISCUSSION

Major risk factor for infection with ESBL producing are long term antibiotic exposure, prolonged ICU stay, severe illness, residence in an institution with high rates of ceftazidime and other third generation cephalosporins use and catheterization (Nathisuwan et al., 2001). The prevalence rate of Klebsiella species recorded in this study is higher than what was reported worldwide by Podschum & Ullman;(1998), Ahmad, (2009) from Shrinagar Kashmir India, Boamponesm et al., (2011) from Ghana, Sarathbabu et al., (2012) from Nellimarla, Andhrapradesh and Chandrakanth et al., (2013) from Gulbarga.

In India reported frequency of ESBL producing Klebsiella species is between 6-87% (Hansotia et al., 1997, Mathur et al., 2005 and Jain et al., 2005). Duttaray and Mehta from Gujrat, India (2005) reported 58% prevalence of ESBL producing K. pneumoniae, isolated from different clinical specimens using PCDDT. Ali et al., 2004 from Rawalpindi Pakistan and Aminzadeh et al., 2008 from south Tehran, Iran reported 40% incidence. The prevalence rate 27.14% was recorded by Basu et al., (2013) from Rajahmundry, Andhrapradesh in respiratory isolates, while Subha & Ananthan, (2002) reported ESBL production 25.8% in various clinical isolates from Chennai, which are higher than our findings. In case of urine and pus isolates, our findings are similar to Shukla et al., (2004) from Aligarh, Uttar Pradesh and higher than the reports of Al-Gerir, (2012) from Mosul, Iraq and Kulkarni et al., (2013) from Pune, India in various clinical Klebsiella isolates. The prevalence of ESBLs varied from one place to other which may be due to infection control measures among different regions.

In the present work, K. pneumoniae was isolated from clinical samples in a higher percentage than the K. oxytoca. Mulvey et al., 2004 and Al-Gerir, 2012 reported the same results. Furthermore there was no significant association between the species and ESBLs formation which was according to Hosoglu et al., 2007. This result may be explained on the basis that ESBLs production is plasmid mediated which is transferred to different bacteria regardless of their species.

Our study demonstrated a high occurrence of ESBL producing Klebsiella species in Amravati and Akola districts. Therefore we suggest, laboratory based surveillance should be conducted on a continuous basis to ESBL producing bacteria. The routine antimicrobial sensitivity tests may fail to detect ESBL mediated resistance against third generation cephalosporins which may lead to treatment failure especially when cephalosporins are used. Therefore it is essential for rapid detection of ESBLs and the indiscriminate use of antibiotics should be discontinued especially with the cephalosporins.

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