

Antimicrobial Resistance and Sensitivity among Isolates of *Klebsiella* spp from Urine Samples in Denizli, Turkey

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To determine the level of resistance to the widely used antibiotics in clinical isolates of *Klebsiella* spp. 15 isolates were collected from special hospital in Denizli and recorded at specimens. Antibiotic resistance was determined by agar disc diffusion method using Mueller-Hinton agar according to Clinical and Laboratory Standards Institute recommendations. The results indicated that sensitivity rate of antibiotics was in the range of meropenem, norfloxacin, piperacillin/ tazobactam (100%), amikacin and ceftriaxone (93%) The most resistant antibiotic was ampicillin(73%). Out of 15 isolates, 3 (20%) isolates showed Multiple Antibiotic Resistance six to nine antibiotics.

Key words: *Klebsiella*, Urine, Antibiotic, Resistance, Multiple.

Urinary tract infection (UTI), considered by the presence of 10⁵ or more bacteria in the urine, impact a great burden in today's clinical practice¹. An considerable amount of risk factors, as hospitalization, underlying medical diseases, immune deficiency indwelling catheters, spinal cord injury and others will help to this conflicting problem^{2,3}. *E.coli*, *Staphylococcus saprophyticus*, *Proteus mirabilis*, *Streptococcus agalactiae* and *Klebsiella* spp are the most common known causes of UTI⁴. *Klebsiella* spp are opportunistic organisms associated with nosocomial infections and is usually isolated from urinary tract, surgical wound, septicaemia and pneumonia¹. They belong to the family Enterobacteriaceae. Strains of *Klebsiella* colonize the mucous membranes of mammals and are found in the epithelia of the nose and pharynx as well as in the intestinal tracts of man⁵. Drug resistance presents an ever-increasing global public health threat that involves all major microbial pathogens and antimicrobial drugs⁶. Bacteria of the genus *Klebsiella* are a common cause of nosocomial infections⁷. There are many studies about antimicrobial susceptibilities of *Klebsiella* isolates in Turkey⁸⁻¹¹.

We aimed in the present study to determine the status of antimicrobial resistance, underlying conditions, and determination of *Klebsiella* spp isolates with from a special hospital in Denizli, Turkey

MATERIALS AND METHODS

Isolation of bacterial strains and identification

15 isolates were collected from hospital patients in Denizli September and October in 2013 and recorded at specimens. Blood agar base and EMB agar (Eosin Metilen Blue) used for *Klebsiella* isolation. Isolates were considered to be presumptive *Klebsiella* spp. gram-negative bacill, mucoid colonies and lactose positive. Confirmation of isolates was performed by using classic chemical tests (motility test, ure hydrolysis, acid production from mannitol, production of H₂S, IMVIC (Indol, Metil Red, Voges-Proskauer & Citrate)^{12,13}.

Antibiotic resistance activity

Antibiotic resistance was determined by an agar disc diffusion test¹⁴ using Mueller-Hinton agar (Difco) according to Clinical and Laboratory Standards Institute¹⁵ recommendations. Twenty different antibiotics were used. For antibiotic resistance determination, the isolates were grown in Luria- Bertani (LB) broth until the turbidity equal

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to the 0.5 Mc Farland standart. Cultures were swabbed on to the Mueller–Hinton agar and all isolates were tested against Meropenem (MEM, 10µg/ml), Piperacillin/ tazobactam (TZP, 110µg/ml), Ampicillin/Sulbactam (SAM, 20µg/ml), Amikacin (AK, 30µg/ml), Ceftazidime (CAZ, 30µg/ml), Tobramycin (TOB, 10µg/ml), Amoxycillin /clavulanic acid (AMC, 30µg/ml), Gentamycin (CN, 10µg/ml), Aztreonam (ATM., 30µg/ml), Cefepime (PM, 30µg/ml), Cefotaxime (CTX, 30µg/ml), Cefuroxime (CXM, 30µg/ml), Ceftriaxone (CRO, 30µg/ml), Sulphamethazol/Trimetoprim (SXT, 25µg/ml), Ciprofloxacin (CIP, 5µg/ml), Cefoperozone (CFP, 75µg/ml), Cephazolin (CZ, 30µg/ml), Norfloxacin (NOR, 10µg/ml), Ampicillin (AM, 10µg/ml), Cefixime (CFM, 5µg/ml).

The isolates those grown in inoculation were evaluated as resistant and the others were evaluated as susceptible¹⁵. The antibiotic discs were dispensed sufficiently separated from each other so as to avoid overlapping of inhibition zones. The plates were incubated at 37°C and the diameters of the inhibition zones were measured after 18 h. All susceptibility tests were carried out in duplicate and were repeated twice if discordant results had been obtained.

Multiple Antibiotic Resistance Index

For all isolates, we calculated the MAR index values (a/b, where a represents the number of antibiotics the isolate was resistant to, b represents the total number of antibiotics the isolates tested against). A MAR index value ≥ 0.2 is observed when isolates are exposed to high risk sources of human or animal contamination, where antibiotics use is common; in contrast a MAR index value ≤ 0.2 observed when antibiotics are seldom or never used¹⁶⁻¹⁷.

RESULTS AND DISCUSSION

The sensitivity of *Klebsiella* spp isolates to antimicrobial agents (n=15) gave high sensitive rates found that *Klebsiella* isolates diffusion tests for meropenem, norfloxacin, piperacillin/tazobactam (100%). Susceptibility of *Klebsiella* isolates were found as 93% for amikacin and ceftriaxone, 87% for Cefepime and Aztreonam, 80% for Cefuroxime, Amoxycillin/clavulanic acid and tobramycin, 73% for Sulphamethazol/Trimetoprim cefoperazone, cefixime and Ampicillin/Sulbactam,

60% for Ciprofloxacin, Ceftazidime. The results were given Table 1.

Many researchers have tested antibiotic susceptibility pattern of *Klebsiella* from clinical samples. Pavani (2012)¹⁸ reported that, 90 % of the *Klebsiella* isolates were sensitive to Amikacin, 36% of the isolates were sensitive to Ciprofloxacin and Norfloxacin, 22% to Cotrimoxazole, 4% to Nalidixic acid and Ampicillin. Bhargavi *et al* (2010)¹⁹ reported that 85.7% sensitivity to Amikacin, Norfloxacin sensitivity of 35 % and Amoxycillin sensitivity of 3.7 % was observed. These findings correlated with Pavani (2012)¹⁸. Both Amoxycillin and Ampicillin belong to the same group of penicillins i.e aminopenicillins. And any one of them can be used as representative drug for testing this group. Therefore the percentage of sensitivity of the isolates to Ampicillin and Amoxycillin is the same. Fluoroquinolone sensitivity of *Klebsiella* isolates

Table 1. Antibiotic resistance pattern of *Klebsiella* spp isolated from urine samples

Antibiotics	Sensitive	Intermediate	Resistance
MEM	15(100%)	(0%)	(0%)
NOR	15(100%)	(0%)	(0%)
TZP	15(100%)	(0%)	(0%)
AK	14(93%)	1(7%)	(0%)
CRO	14(93%)	1(7%)	(0%)
PM	13(87%)	1(7%)	1(7%)
ATM	13(87%)	1(7%)	1(7%)
CXM	12(80%)	(0%)	3(20%)
AMC	12(80%)	2(13%)	1(7%)
TOB	12(80%)	2(13%)	1(7%)
CFM	11(73%)	(0%)	4(27%)
SXT	11(73%)	1(7%)	3(20%)
CEP	11(73%)	2(13%)	2(13%)
SAM	11(73%)	(0%)	4(27%)
CIP	9(60%)	6 (40%)	(0%)
CAZ	9(60%)	6 (40%)	(0%)
CN	8(53%)	7 (47%)	(0%)
CTX	6(40%)	8 (53%)	1(7%)
CZ	5(33%)	8 (53%)	2(13%)
AM	1(7%)	3 (20%)	11(73%)

Meropenem =MEM, Piperacillin/ tazobactam =TZP, Ampicillin/Sulbactam= SAM, Amikacin =AK, Ceftazidime =CAZ, Tobramycin= TOB, Amoxycillin /clavulanic acid =AMC, Gentamycin =CN, Aztreonam =ATM., Cefepime= PM, Cefotaxime =CTX, Cefuroxime=CXM, Ceftriaxone= CRO, Sulphamethazol/Trimetoprim =SXT, Ciprofloxacin=CIP, Cefoprazone=CEP, Cephazolin=CZ, Norfloxacin=NOR, Ampicillin=AM, Cefixime= CFM

Table 2. Multiple antibiotic resistance (MAR) index among 15 *Klebsiella* isolates from urine samples

Source of isolates	Total isolates	MAR index
Female	1	0,43
Female	1	0,38
Female	1	0,29
Male	1	0.14
Female	1	0,09
Female	4	0,004
Female	1	0,004
Male	2	0
Female	3	0
Total	15	

in his study was 36 % in our study showing only a moderate to low susceptibility (Pavani, 2012)¹⁸. Sekowska *et al.* (2011)²⁰ reported that, flouroquinolones are effective drugs for treating infections caused by *Klebsiella* species. Arsalan *et al* (2014)²¹ reported that in the *Klebsiella* species, 71.42% and 64.29% resistance to cefazolin and cefuroxime respectively, was observed. Aminoglycosides such as gentamycin and tobramycin were found to be more susceptible to all the clinical isolates. Quinolones like ofloxacin and enoxacin were showed good sensitivity to nearly all the clinical isolates. In south-western Nigeria Okesola and Makanjula (2009)²² reported that there was an increased isolates to cefotaxime as compared to ceftazidime. 69.3% of *Klebsiella* isolates were resistant to cefotaxime as compared to 45.2 % resistance to ceftazidime. This is in keeping with the findings of Kumar *et al.* (2006)²³ where 85% of *K.pneumoniae* were resistant to cefotaxime but only 37% Ceftazidime. Malakan-Rad and Momtazmanesh (2004)²⁴ reported that among the four tested antibiotics ampicillin is the most sensitive (100%), amikacin is the least sensitive (46%). The sensitivity rate were 69% for ceftriaxone and 73% for gentamicin. Egbebia and Famurewa (2011)²⁵ reported that ceftazidime, aztreonam and cefotaxime showed no susceptibility rate but, ceftriaxone showed 2.4% effect of selected third generation cephalosporins on some *Klebsiella* species. Similarly, Patel *et al.* (2009)²⁶ reported the resistance of *Klebsiella* from Tertiary Care Unit in Gujurat, to Cefoperazone, cefotaxime and ceftriaxone. The use of third generation

cephalosporin with \pm -lactamase inhibitors such as clavulanic acid, sulbactam or tazobactam would reduce the resistance posed by *Klebsiella* spp. This was confirmed by the works of Ivanov (2006)²⁷ who reported that lowest MICs were observed with use of inhibitor-protected agent such as combination of ceftazidime/clavulanic acid. Similarly, Patel *et al.* (2009)²⁶ reported that addition of sulbactam to cefoperazone, cefotaxime and ceftriaxone monotherapy significantly reduced the percentage resistance and increased the percentage susceptibility against *Klebsiella*.

Among the *Klebsiella* isolates from urine samples, the lowest MAR index value was 0, the highest MAR index value was 0.43. MAR index were given Table 2. Out of 15 isolates 3(20%) isolates showed Multiple Antibiotic Resistance six to nine antibiotics. These figure were lower than was seen in the series done by Osundiya *et al* (2013)²⁸ who also reported that MAR index value of isolates was 66.7%. This may be due to either the difference in the definition used for multidrug resistance in both works or may be an indication of a significant jump in the emergence of multidrug strains.

Knowledge of the local antimicrobial susceptibility pattern is the empirical treatment of outbreaks of infections caused by *Klebsiella* species. For example, it could be said that meropenem, norfloxacin, piperacillin/ tazobactamin that order could be used in the empiric treatment of infections caused by *Klebsiella* species from urine samples.

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