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COMPARISON OF ANTIBIOTIC SENSITIVITY RATIO OF *ESHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* STRAINS ISOLATED FROM VARIOUS CLINICAL SPECIMENS IN INTENSIVE CARE UNIT

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ABSTRACT

A retrospective analysis of the widely used antibiotics all susceptibility testing results from *Esherichia coli* and *Klebsiella pneumoniae* cultured from clinical specimens Muğla Sıtkı Koçman University Educatioan and Research hospital (January to December in 2015) was performed. The new BD PHOENIX automated microbiology system (Becton Dickinson Diagnostic Systems, Sparks, Md.) is designed for automated rapid antimicrobial susceptibility testing and identification of clinically relevant bacteria. Minimum Inhibitory Concentration (MIC) results previously obtained in recent clinical isolates with well-defined in isolates with well-characterized resistance mechanisms with the microdilution method were re-interpreted for the susceptible, intermediate and resistant categories using the 2012 EUCAST breakpoints. Clinical samples are most commonly isolated from tracheal aspirates, wound site, blood, mucus, abcess, catheter, urine samples, throat and nose cultures. *E.coli* isolates were highly resistant to Ciprofloxacin (CIP), Cefepime (FEP), and Aztreonam (ATM) 47%, %47 and 40% respectively. Unlike *K.pneumoniae* isolates were highly resistant to Ampicillin/Sulbactam (SAM), ATM and FEP 58%, 53% and 50% respectively. Both of the bacteria showed the highest sensitivity rate to Amikacin (AN) 98% and 90% *E.coli* and *K.pneumoniae* respectively. Out of the 60 *E.coli* strains 34 (57%) isolates showed Multiple Antibiotic Resistance (MAR) two to ten antibiotics. Out of the 60 *K.pneumoniae* strains 38 (63%) isolates showed MAR two to ten antibiotics. Considering the antibiogram, AN and Meropenem (MEM) should be preferred drugs for *K. pneumoniae* and *E.coli* infection isolated from clinical samples.

KEYWORDS:
Esherichia coli, *Klebsiella pneumoniae*, antibiotic sensitivity, clinical specimens, intensive care unit

INTRODUCTION

Antibiotic resistance in Gram-negative bacteria is a major threat to public health [1-4]. Patients with non-severe infections caused by multidrug-resistant bacteria are subject to in-hospital intravenous therapy because there are no effective oral drugs available. Resistance to empirical antibiotic therapy results in delayed appropriate antibiotic treatment for severe infections, which is associated with increased mortality, prolonged hospital stay and higher costs [5-7]. Further, resistance challenges the achievements of modern medicine, including advanced surgery and immunosuppressive treatment, which are dependent on effective antibiotics.

Escherichia coli and *Klebsiella pneumoniae* are two important members of Gram-negative rods (Enterobacteriaceae), which belongs to a part of human gastrointestinal normal flora [8-10]. These organisms are substantial human pathogens which lead to a wide spectrum of hospital and community-acquired infections such as urinary tract infection (UTI), septicemia, pneumonia, peritonitis, meningitis, etc. [11, 12]. In the lack of appropriate treatment of infections by these organisms, noticeable morbidities and mortalities will occur [13].

The aim of this study was to determine the characteristics and patterns of antibiotic resistance among isolates of *Escherichia coli* and *Klebsiella pneumoniae* recovered from clinical specimens in Muğla

MATERIALS AND METHODS

Bacterial isolates. Ethical approval is was taken before study. Because of retrospective analysis, we did not patient approval. 60 *Escherichia coli* and 60 *Klebsiella pneumoniae* were isolated from clinical specimens from intensive care unit of internal medicine in Muğla Sıtkı Koçman University Education and Research hospital. Bacterial isolates were identified to level of species and subspecies by using the morphological and traditional biochemical tests and automatic diagnostic systems currently present in the market and commonly used for AST (Antimicrobial Susceptibility Testing) in clinical laboratories will therefore have to incorporate these criteria in their instruments to meet the needs of European microbiology laboratories according to standard methods described by [14-16]. All isolates were obtained from patients at intensive care units. In total, 120 *Escherichia coli* and *Klebsiella pneumoniae* were isolated from various clinical samples and detected by the PHOENIX (Becton Dickinson, USA) at the microbiology laboratory of our hospital between from January to December 2015.The PhoenixTM Automated Microbiology System (BD Diagnostics, Sparks, MD, USA) is designed for the rapid bacterial identification at the species level and determination of AST of clinically significant human bacterial pathogens [17].

Antibiogram profile of *Escherichia coli* and *Klebsiella pneumoniae.* Minimum Inhibitory Concentration (MIC) results previously obtained in recent clinical isolates with well-defined in isolates with well-characterized resistance mechanisms with microdilution method were re-interpreted for the susceptible, intermediate and resistant categories using the 2012 EUCAST breakpoints. Ten different antibiotics were used.

Multiple Antibiotic Resistance (MAR) 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 isolate 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 <or = 0.2 observed when antibiotics are seldom or never used [18-19].

RESULTS AND DISCUSSION

Resistance pattern results. The results of resistance pattern of *E.coli* isolates in our locality to antimicrobial agents showed that the 60 *E.coli* and 60 *K.pneumoniae* strains tested against ten antimicrobial agents in Table 1. *E.coli* isolates were highly resistant to Ciprofloxacin (CIP), Cefepime (FEP), and Aztreonam (ATM) 47%, %47 and 40% respectively. Unlike *K.pneumoniae* isolates were highly resistant to Ampicillin/Sulbactam (SAM), Aztreonam (ATM) and Cefepime (FEP) 58%, 53% and 50% respectively. Both of the bacteria showed the highest sensitivity rate to Amikacin (AN) 98% and 90% *E.coli* and *K.pneumoniae* respectively.

In our study, when we compared to resistance of CIP, *E.coli* isolates showed high antibiotic resistance with 47% CIP. Unlike *K.pneumoniae* isolates showed resistance to CIP with 18%. Many researchers were reported resistance rate to CIP *E.coli* and *K.pneumoniae* [20-22]. Our results were similiar to Giray et al. (2012) [23] who also reported that Ciprofloxacin resistance rate to *E.coli* was 47%.

In this study, resistance of FEP *E.coli* and *K.pneumoniae* 47% and 50% respectively. Many researchers were reported resistance rate to FEP *E.coli* and *K.pneumoniae* [24-27]. Our results were similiar to previous researchers.

When it comes to resistance of ATM *K.pneumoniae* isolates showed high resistance to ATM with 53%. Unlike *E.coli* isolates showed resistance to ATM with 40%. Many researchers were reported resistance rate to ATM *E.coli* and *K.pneumoniae* [25-33]. Our results were similiar to Sabir et al. (2014) [33] who also reported that ATM resistance rate to *E.coli* was 44.8%.

In our study, resistance of Norfloxacin (NOR) were *E.coli* with 35% and *K.pneumoniae* with 32%. Many researchers were reported resistance rate to NOR *E.coli* and *K.pneumoniae* [34-36].Our results were similiar to previous researchers.

TABLE 1
Antibiotic resistance pattern of *E.coli* and *K.pneumoniae* isolated from clinical specimens in intensive care unit.

Antibiotics	*E.coli*			*K.pneumoniae*		
	R	**I**	**S**	**R**	**I**	**S**
CIP	28(47%)	-	32(53%)	23(18%)	1(2%)	36(60%)
FEP	28(47%)	2(3%)	30(50%)	30(50%)	1(2%)	29(48%)
ATM	24(40%)	-	36(60%)	32(53%)	-	28(47%)
NOR	21(35%)	-	39(65%)	19(32%)	1(2%)	40(67%)
CXM	20(33%)	1(2%)	39(65%)	28(47%)	1(2%)	31(52%)
SAM	18(30%)	14(23%)	28(47%)	35(58%)	5(18%)	20(33%)
TZP	6(10%)	4(7%)	50(83%)	14(23%)	7(12%)	39(65%)
AMC	5(8%)	8(13%)	47(78%)	13(22%)	9(15%)	38(63%)
MEM	3(5%)	2(3%)	55(92%)	15(25%)	3(5%)	42(70%)
AN	1(2%)	-	59(98%)	6(10%)	-	54(90%)

Abbrevations; CIP; Ciprofloxacin, FEP; Cefepime, ATM; Aztreonam, NOR; Norfloxacin, CXM; Cefuroxime sodium, SAM; Ampicillin/Sulbactam; TZP;Tazobactam/Piperacillin, AMC; Amoxicillin/Clavulanic Acid, MEM; Meropenem, AN; Amikacin, -; No result.

TABLE 2
Number of clinical samples and MAR Index 60 *E.coli* and *K.pneumoniae* strains.

Clinical Samples in Intensive Care Unit	*E.coli*		*K.pneumoniae*	
	Number of Isolates	MAR	Number of Isolates	MAR
Urine	37	0(13isl), 0,1(4isl), 0,2(7isl), 0,3(1), 0,4(2isl), 0,5(3isl), 0.6(4isl), 0,7(1), 0,8(2isl)	35	0(9isl), 0,1(4isl), 0,2(3isl), 0,3 (3isl), 0,4(3isl), 0,5(1isl), 0,6 (5isl), 0,7(3isl), 0,8(1isl), 0,9(2isl), 1(1isl)
Tracheal aspirate	6	0(1), 0,1(1isl), 0,5(2isl), 0,6(1isl), 0,7(1isl)	10	0(4isl), 0,2(2isl), 0,3(1isl), 0,4(1isl), 0,5(1isl), 0,8(1isl)
Blood	4	0,3(2isl), 0,4(2isl)	5	0(2isl), 1(1isl), 0,5(1isl), 0,9(1isl)
Gaita	1	0,4	-	
Vagen	6	0(2isl), 0,1(3isl), 0,2(1isl)	2	0(1isl), 0,5(1isl)
Abces	4	0(1isl), 0,1(1isl); 0,2(1isl); 0,3(1isl)	2	0(1isl), 0,5(1isl)
Wound	1	0,3(1isl)	3	0,1(1isl), 0,8(1isl),1(1isl)
Periton	1	0,3(1isl)	-	
Throat	-	-	1	0,4(1isl)
Mucus	-	-	2	0,4(1isl), 0,7(1isl)

MAR, Multiple Antibiotic Resistance Index, isl; isolates, -; No result.

When we compared to resistance of Cefuroxime sodium (CXM) *K.pneumoniae* isolates showed high resistance to CXM with 47%, but *E.coli* isolates showed resistance of CXM with 33%. Many researchers were reported resistance rate to CXM *E.coli* and *K.pneumoniae* [30, 37]. Our results were similiar to previous researchers.

In this study, *K.pneumoniae* resistance (58%) of SAM were higher than *E.coli* (30%). Many researchers were reported resistance rate to SAM *E.coli* and *K.pneumoniae* [38-42]. Our results were similiar to Tsakris et al. (1997) [42] who also reported that SAM resistance rate to *E.coli* was 32.1% .

When we compared to resistance of Tazobactam/Piperacillin (TZP), *K.pneumoniae* (23%) isolates showed more resistance than *E.coli* (10%). Many researchers were reported resistance rate to TZP *E.coli* and *K.pneumoniae* [43, 44]. Our results were similiar to Tsakris et al. (1997) [42] who also reported that TZP resistance rate to *E.coli* was 10.5%.

When it comes to resistance of Amoxicillin/Clavulanic acid (AMC), *K.pneumoniae* (22%) isolates showe more resistance than *E.coli isolates* (8%). Many researchers were reported resistance rate to AMC *E.coli* and *K.pneumoniae* [45-47]. Our results were similiar to previous researchers.

In this research, Meropenem (MEM) and AN were the most sensitive antibiotics to both of the isolates. While percentage of MEM sensitivity was 92% and 70% *E.coli* and *K.pneumoniae* respectively. Many researchers were reported resistance rate to MEM *E.coli* and *K.pneumoniae* [36, 48-50]. Our results were similiar to Sohail et al. (2015) [51] who also reported that MEM resistance rate to *E.coli* was 3%.

In our study, sensitivity rate of AN were *E.coli* with 98% and *K.pneumoniae* with 90%. Many researchers were reported resistance rate to AN *E.coli* and *K.pneumoniae* [23,31,52-54]. Manikandan and Amsath (2013) [55] reported that the highest susceptibility pattern of bacterial isolates from respiratory tract infection showed to AN like our study.

MAR index results. Out of the 60 *E.coli* strains 34 (57%) isolates showed MAR two to ten antibiotics. 17 (28%) isolates showed no antibiotic resistance especially isolated from urine samples. Out of the 60 *K.pneumoniae* strains 38 (63%) isolates showed MAR two to ten antibiotics. Both of the bacteria, 17 (28%) isolates showed no antibiotic resistance especially isolated from urine samples. The MAR indices give an indirect suggestion of the probable source(s) of the organism. The results were given Table 2. Some researchers have reported MAR rate to *E.coli* from 2% to 97% [56-58]. MAR is considered as a good tool for risk assessment. This also gives an idea of the number of bacteria showing antibiotic resistance in the risk zone in the study's routine susceptibility testing. This MAR index also recommended that all isolates, somehow, originated from the environment where antibiotics were over used% [59]. Notably, MAR indices for the clinical isolates of *E. coli* were generally lower than *Klebsiella pneumoniae* isolates recovered from same conditions (Table 2).

CONCLUSIONS

High antibiotic resistance of *K. pneumoniae* and *E.coli* towards commonly used antibiotics are the major reasons for prolonged infections, increased hospitalization, increased cost of therapy and enhanced morbidity and mortality rates. *K. pneumoniae* and *E.coli* was found to be most sensitive to AN and MEM. Considering the antibiogram, AN and MEM should be preferred drugs for *K. pneumoniae* and *E.coli* infection isolated from clinical samples. MAR indices for the clinical isolates of *E.*

coli were generally lower than *K. pneumoniae* isolates recovered from same conditions. The findings in present the study suggest that there is an urgent need for constant monitoring of susceptibility of pathogens in different populations to commonly used antimicrobial agents. The data of this study may be used to determine trends in antimicrobial susceptibilities, to formulate local antibiotic policies and overall to assist clinicians in the rational choice of antibiotic therapy to prevent misuse, or overuse, of antibiotics.

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