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Screening of Urinary Tract Bacterial Infections and Their Antibiogram Among Non-Pregnant Women Admitted to Al-Sadiq Hospital, Iraq

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ABSTRUCT: Infections of urinary tract have been proven to be the most encountered bacterial infections in human. Our study was oriented toward isolation and diagnosis of bacterial pathogens involved in causation of urinary tract infection in community with detection of antibiogram in non-pregnant females with careful determination the antibiotic of choice to treat them. Urine samples (125) from mid stream were collected aseptically which cultivated on various media. Precise diagnosis and antibiogram for all uropathogens were done with the highly sensitive and accurate device (Vitek 2 Compact System). Out of 125 urine samples enrolled, an overall of 56 (44.8%) samples were exhibited a positive growth for bacteria. Positive bacterial growth was significantly associated with age ($P < 0.05$) and UTI was mostly encountered in age group of 18-22 years (30.4%) while age group of 23-27 years showed the lower tendency toward UTI (8.9%). Of the 56 positive female patients,46 (82.1%) patients were symptomatic bacteriuria and 10 (17.9) were asymptomatic. The major uropathogen isolated was *Escherichia coli* (33.9%), then *Streptococcus pyogens* which constituted (25%). Twenty one isolates (84%) of Gram-positive uropathogens exhibited sensitivity to vancomycin and 20 isolates (80%) showed sensitivity to nitrofurantoin. Thirty isolates (96.8%) of Gram-negative uropathogens exhibited sensitivity to ertapenem and 24 isolates (77.4%) showed sensitivity to norfloxacin. The majority of uropathogen involved in causation of UTI is *Escherichia coli*. Antimicrobial susceptibility testing is an essential to achieve the desired antibiotic with excellent efficacy.

Key words: Urinary tract infection, Non-pregnant women, Al-Sadiq Hospital.

INTRODUCTION

The most common outpatient infections are urinary tract infections (UTI) and considered as some health-related problem around the world [1]. Predicting the possibility of UTI occurrence through symptoms and test results may be complex [2]. Different age groups, both male and female are susceptible for UTI but the higher incidence in female other than male [3] related to urethra of female and its closeness to area of anus besides the activity of hormones [4].

UTI is considered as a serious disease in human because of frequent recurrence, hardness in removal and prolonged treatment duration. Risky individuals for developing UTI involve newly born babies (especially the premature one), mature female, sexually active women and even the elderly women [5]. Bacterial infections involving both Gram negative bacteria (GNB) and Gram positive bacteria (GPB) are the primary causative agents included in the occurrence of UTI throughout the human lifetime. In addition to that, some fungi may participate in the development of UTI [6].

UTIs are considered as the second most popular infections occurring through community field and are classified as complicated and uncomplicated [7]. The most common symptoms which generally associated with

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UTI are: fever, chills, dysuria, urination urgency, frequency, burning, strong smell, flank pain and supra-pubic pain [8].

It is recorded that there is increasing in multidrug resistance among uropathogenic bacteria which considered as an important and comprising a problem to public health with difficulties in eradication [9]. Consequently, bacterial pathogens causing UTI are evolving and acquiring various mechanisms of antibiotic resistance, exhibiting insensitivity to more than two antibiotics and this will render the physician that followed the case with little antibacterial options for treating UTI. The evolution of antimicrobial insensitivity (particularly to antibiotic) in controlling UTI is regarded as huge problem toward the health of people, especially in developing countries [10], in which a poor personal hygiene, an elevated degree of poverty, in addition to use drugs without prescription are collectively being an aggravating factors [11].

The current study was conducted for determining the common bacterial pathogens causing UTI in community with susceptibility pattern identification in different age groups of women visited to an outpatient department of hospital using a newer and precise technique. The sensitivity of isolated pathogens to different antibiotics was also performed to detect the drug of choice reliable to treat it.

MATERIALS AND METHODS

Study Population

The study was performed in a Al-Sadiq tertiary care unit in Babylon Province, Iraq. The study was happened through the period from 25 / December / 2019 to 15 / March / 2020, involving female patients visited various clinical specialties in the outpatient departments (OPDs) of the hospital (gynecology, surgery, internal medicine). A total of 125 urine samples were enrolled in this study of female with clinical suspicion to have UTI whether asymptomatic or have some or all of the following clinical signs and symptoms including: fever, chills, passing frequent urine with small amount, burning sensation, persistent urge to urinate, strong-smelling urine, flank pain and supra-pubic pain.

Five age groups were included in this study, 25 female suspected individuals in each group. All needed information were recorded involving: name, age, treatment and clinical history, whether pregnant or not. The five age groups included in the study were group (18 year – 22 year), group (23 year – 27 year), group (28 year – 32 year), group (33 year – 37 year) and group (38 year – 42year).

Exclusion Criteria

In the current study, females with history of admission to the hospital of 2 weeks before their visiting to the OPDs, pregnant females and females on menstrual cycle were excluded from this study. Additionally, females with any known anomalies in the urinary tract and females taking courses of antibiotics were also excepted from current study.

Clinical Specimens

In this study, all patients were educated about how to collect the specimen of urine aseptically within a sterile plastic wide-mouth, leak-proof containers. Each patient was asked about the collected urine specimen to ensure that all specimens were obtained from mid-stream urine to avoid contamination. After that, the urine specimens were labeled and sent directly for investigation in the laboratory.

Bacterial Pathogens Isolation

All samples of urine collected were inoculated on various sterile culture media (MacConkey agar , Blood agar, Nutrient agar, Chocolate agar). The inoculated agar plates were incubated at 37 °C for 24-48 hours. Then, all cultured plates were tested to indicate whether there is microbial growth or not, and if growth was present, morphological features was observed (including colony morphology, arrangement, odor, color). Consequently, the bacterial cultures were subjected for gram staining technique to find out whether the obtained isolates is gram-positive or gram-negative [12].

Idenification of Uropathogens and Antibiogram

The precise identification of uropathogens with their antibiogram (antimicrobial susceptibility testing) were conducted using an automated, precise device which is the Vitek 2 Compact System (Biomerieux company, France). In case of primary diagnosis of Gram-negative bacteria, both Vitek 2 GN ID Card (Gram-Negative Identity Card) with Vitek 2 AST Card (Antimicrobial Susceptibility Testing Card) specific for Gram-negative bacteria were used. When the primary diagnosis revealed Gram-positive bacteria, both Vitek 2 GP ID Card (Gram-Positive Identity Card) with Vitek 2 AST Card specific for Gram-positive bacteria were used.

Different classes of antibiotics (set of antibiotics for Gram-positive isolates and other set for Gram-negative isolates) were used in the present study. The antibiotic classes used in the study like β-lactam antibiotics, aminoglycosides, macrolides, tetracyclines, quinolones, anti-folate and others.

Statistical Analysis

In the current study, SPSS V-27 was used for analyzing the data. The statistical test used in the study was the Chi-square test that used to determine the dependency between variables. P values less than 0.05 ($P < 0.05$) were considered to be statistically different.

Ethics Statement

Consents were obtained from all patients included in the study concerning all aspects of the study as a regular care for patients in the tertiary care hospital. All information regarded the patients in the study were preserved in order to protect patients privacy. The obtained isolates were numbered, stored properly for study and treatment purposes. The study protocol was reviewed and confirmed by the Committee of Medical Ethics in Iraq which is known as Institutional Review Board (IRB) and in accordance with ethical standards of Declaration of Helsinki.

RESULTS AND DISCUSSION

The current study involved five different age groups. Each group included 25 female suspected individuals as illustrated in Table 1. Many obstacles encountered through the period of study in achieving an equal number of suspected individuals in each group. In spite of these impediments, this was reached since it is necessary to achieve a realistic comparison among various age groups in the current study.

Urinary tract infection (UTI) is a common complaint in an outpatient, whether specialist clinics or even primary care clinics. It is important for the clinicians to have a comprehensive understanding about the physiology, pathophysiology, epidemiology and treatment strategies of UTI [13]. UTIs are a significant health-care problem worldwide, mostly appeared in outpatient clinic departments as well as in hospitalized patients. The estimated incidence of UTIs was about 150 million new cases every year [14].

In the study, out of 125 urine samples from females cultivated for detection presence of microbial growth, 56 (44.8%) urine samples showed positive microbial growth. Among the various age groups in the study, age group (18 year - 22 year) was exhibited the highest vulnerability for UTI (30.4 %) followed by age group (28 year - 32 year) (25 %) while age range (23 year - 27 year) showed least percentage of bacterial growth (8.9 %) and this was best summarized in Table 1.

TABLE 1. Positive bacterial growth among various female age groups.

Age Group	No. of each Group	No. of Negative Urine Culture	No. of positive Urine Culture	Percentage of Positive Culture
18 year - 22 year	25	8	17	30.4 %
23 year – 27 year	25	20	5	8.9 %
28 year – 32 year	25	11	14	25 %
33 year – 37 year	25	14	11	19.6 %
38 year – 42 year	25	16	9	16.1 %
Total Number	125	69 (55.2 %)	56 (44.8 %)	100 %

The current study was conducted to supply a valuable information about the uropathogens causing community-acquired UTIs (for both symptomatic and asymptomatic non-pregnant women) and illustrated in details the susceptibility testing (sensitivity / resistance) to various classes of antibiotics, so make physician oriented in the selection of appropriate antimicrobial agents. A relatively higher occurrence of UTIs (44.8%) among female patients included in the present study is related to the female urethra which is shorter than that of male. In the study, the occurrence of community-acquired UTIs in various age groups of women were significantly related and dependent, this is apparent from our findings which showed that most uropathogens were recovered from urine of age between 18 to 22 years and this was in accordance with other study in Uganda, who found that female with

age ≤19 years had statistically significant relationships with UTI (p value < 0.05) [15]. This may be attributed to many factors like geographical location (some female patients may be coming from rural regions), personal hygiene (illiterate females), hormonal abnormality and others. Other study in India, reported that UTI was more prevalent in female with age from 15 to 44 years in comparison with age above 45 years [16].

The age group was found to be significantly related with the occurrence of positive bacterial growth with P values less than 0.05 (P < 0.05), meaning that both variables were dependent. Among the 56 female patients with positive urine culture obtained, 46 (82.1%) female patients were recorded as symptomatic bacteriuria (with various signs and symptoms) and 10 (17.9%) were asymptomatic bacteriuria (without signs and symptoms). The distribution of symptomatic and asymptomatic female patients according to various age groups was illustrated in Table 2. This may be related to many factors like gender, age, sexual behavior, marital status, hormonal factors. In another study, they were recorded that 36 patients (10.7%) with asymptomatic bacteriuria [17].

TABLE 2. Distribution of symptomatic and asymptomatic bacteriuria in relation to age groups

Age Group	No. of positive Urine Culture	Symptomatic Bacteriuria	Asymptomatic Bacteriuria
18 year - 22 year	17	13	4
23 year – 27 year	5	3	2
28 year – 32 year	14	11	3
33 year – 37 year	11	10	1
38 year – 42 year	9	9	0
Total Number	56 (100%)	46 (82.1 %)	10 (17.9 %)

The current study revealed different types of bacterial pathogens (uropathogens) recovered from various urine samples of female patients. Various types of both Gram-positive and Gram-negative bacterial isolates were obtained from these urine samples. Out of 56 bacterial isolates obtained, 31 isolates (55.4%) were found to be Gram-negative bacterial isolates with 25 isolates (44.6%) were diagnosed as Gram-positive bacterial isolates. It was found that, Escherichia coli (abbreviated E. coli) (Gram-negative bacteria) were the predominant uropathogens (19 isolates of the total 56) recovered from urine samples, it constituted (33.9%) of the total isolates obtained in the study. Furthermore, 14 isolates (25%) were found to be Streptococcus pyogens which represented the second most uropathogens causing UTIs while pseudomonas aeruginosa constituted the lowest percentage (1.8%), only one isolates, among other bacterial isolates recovered as illustrated in "Table 3". The different types of bacterial isolates obtained and their percentages were clearly illustrated in Figure1. Moreover, it was obviously reported that the predominant causative agent of UT

Is concerning age 18-32 years was E. coli while age group (38-42 year) presented with Streptococcus pyogens as the most common causative agent. In the study, it was noticed that E. coli was the most common agent isolated from both symptomatic and asymptomatic bacteriuric patients.

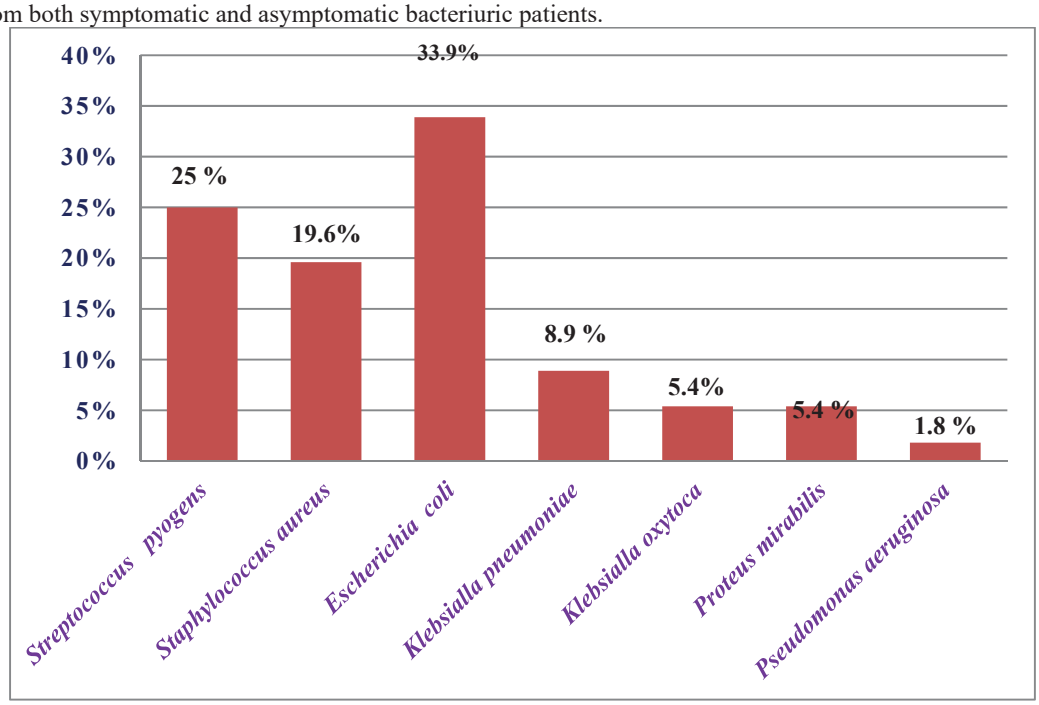

FIGURE 1. Percentages of uropathogens recovered in the current study

This figure shows the different types of uropathogens obtained in the study and illustrates the higher percentages of *Escherichia coli* among others followed by *Streptococcus pyogens* while the lowest percentage is represented by *pseudomonas aeruginosa*.

TABLE 3. Various kinds of bacterial pathogens obtained in current study.

Types of isolates	Age Groups					Total Isolates No.
	18 year - 22 year	**23 year - 27 year**	**28 year - 32 year**	**33 year - 37 year**	**38 year - 42 year**	
Staphylococcus aureus	3		2	3	3	11
Streptococcus pyogens	3	1	3	3	4	14
Proteus mirabilis	1		1	1		3
Klebsialla oxytoca			1	1	1	3
Klebsialla pneumoniae	2	1	2			5
Pseudomonas aeruginosa	1					1
Escherichia coli	7	3	5	3	1	19
Total Number	17	5	14	11	9	56

E. coli was the most frequently isolated bacteiuric pathogen and this may be attributed to the fact that there are abundant fecal distribution of mostly E. coli and because of close vicinity of anal orifice from the female urethral meatus, so this together will increase the chance of E. coli to cause UTIs in female. Uropathogenic E. coli characterized by many virulence factors that enable them to colonize the urinary tract and to resist the different host defense mechanisms [18]. Virulence factors of E. coli that have been potentially involved in establishment of UTIs including bacterial surface adhesins (fimbriae) which of different types that mediate attachment to tissue of urinary tract and subsequent colonization (adhesins are important determinant of pathogenicity that promote bacterial invasion [19]. Furthermore, the second important virulence factor is the toxins which are important in E. coli - mediated diseases, toxin production by urinary tract colonizing E. coli can cause an inflammatory response that can lead to UTIs symptoms [20].

Many studies reported that E. coli was the primary cause of UTIs. In Poland, Stefaniuk and his colleagues recorded that E. coli was most frequently isolated pathogen from both complicated and uncomplicated UTIs which constituted 71.4% of the total isolates [21]. In another study, they were revealed that E. coli was the predominant isolated pathogen (25.3%) followed by S. aureus (20.3%) but S. pyogens (7.6%) (22) while our study, although reported that E. coli was the most common pathogen isolated (33.3%), but the second pathogen isolated was Streptococcus pyogens (24.6%) followed by S. aureus (21%).

Antimicrobial susceptibility pattern (antibiogram) was conducted including all Gram-positive and Gram-negative bacterial isolates (56 isolates). Table 4 and Table 5 represented antimicrobial susceptibility pattern for Gram-positive and Gram-negative bacterial isolates, respectively. Bacterial isolates exhibited different levels of susceptibility (resistance or sensitivity) to the selected antibiotics (10 antibiotics) enrolled in the current study. Some isolates showed an elevated level of resistance to antibiotics and may be considered as multidrug or even extensively drug resistant isolates as in isolate NO.5, NO.17, NO.20, NO.34, NO.40 and NO.55. Concerning Gram-positive isolates (table 4), the percentage of resistance to antibiotics was ranged from the lowest value 10% (isolate No.13, *Streptococcus* pyogens) reaching to the highest value which was 80% (isolate No.17, Staphylococcus aureus). Of these 25 Gram-positive isolates, 21 (84%) bacterial isolates were found as sensitive to the antibiotic vancomycin in addition to 20 (80%) bacterial isolates were recorded as sensitive to the antibiotic nitrofurantoin.

Antimicrobial susceptibility patterns of Streptococcus pyogens and S. aureus (obtained in the study) revealed various behavior toward the different classes of antibiotics used in the study (10 antibiotics) with better sensitivity of 20 (80%) bacterial isolates to nitrofurantoin antibiotic (available orally) which make it preferred antibiotic to treat mild, uncomplicated community-acquired UTI due to Gram-positive bacteria while 21 (84%) bacterial isolates showed good sensitivity to vancomycin antibiotic (parenteral administration) that can be used to treat moderate to severe, complicated community-acquired UTI due to Gram-positive bacteria. On the other hand and concerning Gram-negative bacteria recovered in the study, it was found that 24 (77.4%) bacterial isolates showed

better sensitivity to the antibiotic norfloxacin (orally used) which can be prescribed to treat mild, uncomplicated community-acquired UTI due to Gram-negative bacteria while 30 (96.8%) bacterial isolates appeared very excellent sensitivity to the antibiotic ertapenem (parentally used) which can be selected to overcome moderate to severe, complicated community-acquired UTI caused by Gram-negative bacteria. In another study, they reported a higher percentage of antimicrobial sensitivity for uropathogens (especially Gram-negative) toward carbapenem antibiotics (Meropenem, MRP; Imipenem, IMP) as follow: E. coli (MRP: 95.45% and IMP: 98.89%), Pseudomonas aeruginosa (MRP: 100% and IMP: 95%), Proteus spp. and Enterobacter spp. (MRP: 100% and IMP: 100%) [23].

About Table 5 which concerned with Gram-negative bacterial isolates, the percentage of resistance to antibiotics was ranged from 0%, means 100% sensitive to antibiotics, which represented the lowest degree of resistance (isolate No.29, E. coli) reaching to 80% of resistance (isolate No.55, Proteus mirabilis) which considered the highest degree of resistance among other Gram-negative isolates obtained in the study. Furthermore, out of 31 Gram-negative isolates obtained, it was observed that 30 (96.8%) bacterial isolates were found as sensitive to the antibiotic ertapenem in addition to 24 (77.4%) bacterial isolates were recorded as sensitive to the antibiotic norfloxacin.

In related study, it was reported that nitrofurantoin was the most effective medication for treating all uropathogens obtained from urine of non-pregnant women who attended to Mulago Hospital with lower sensitivity recorded for the SXT antibiotic. The high rate of resistance to ciprofloxacin, SXT and ampicillin may prevent the utilization of these widely prescribed medications in empiric treatment of urinary tract infection in Uganda [24].

TABLE 4. Antibiotic susceptibility testing of Gram positive uropathogens

Types of bacteria isolates	Isolate numb	Antibiotic susceptibility pattern										% of bacterial resista
		PTZ	CTR	CPM	ETP	NA	NOR	TOB	AM	MNO	SXT	
Escherichia coli	26	S	R	S	S	S	S	S	R	S	S	20%
Escherichia coli	27	S	R	S	S	S	R	S	R	R	R	50%
Escherichia coli	28	R	S	S	S	R	S	S	S	R	S	30%
Escherichia coli	29	S	S	S	S	S	S	S	S	S	S	0%
Escherichia coli	30	S	R	S	S	S	S	S	S	R	S	20%
Escherichia coli	31	S	S	R	S	R	S	R	S	S	R	40%
Escherichia coli	32	R	S	S	S	R	S	S	R	R	S	40%
Escherichia coli	33	S	R	S	S	S	S	S	S	S	S	10%
Escherichia coli	34	R	R	R	S	R	R	R	S	R	R	80%
Escherichia coli	35	S	S	S	S	R	S	S	R	S	S	20%
Escherichia coli	36	S	R	S	S	R	S	S	R	S	S	30%
Escherichia coli	37	S	S	R	S	R	S	S	S	R	S	30%
Escherichia coli	38	R	R	S	S	S	R	S	R	S	R	60%
Escherichia coli	39	R	R	S	S	S	S	R	R	R	S	50%
Escherichia coli	40	R	R	R	S	R	R	S	S	R	R	70%
Escherichia coli	41	S	R	R	S	S	S	R	S	S	S	30%
Escherichia coli	42	R	S	S	S	R	S	S	S	S	R	30%
Escherichia coli	43	S	R	R	S	S	S	S	S	S	S	20%
Escherichia coli	44	R	S	S	S	S	S	R	R	R	S	40%

CONCLUSION

E. coli is the predominant uropathogen involved in the causation of community-acquired UTIs among female patients with different age groups followed by Streptococcus pyogens. Antimicrobial susceptibility pattern is of important value for physicians which facilitate in proper selection of antibiotic. Our study revealed the higher effectiveness of both nitrofurantoin and vancomycin antibiotics against Gram-positive bacterial isolates whereas both norfloxacin and ertapenem antibiotics are demonstrated to have potent effect toward Gram-negative bacterial isolates obtained in the current study.

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TABLE 5. Gram-negative bacterial isolates

Types of bacterial isolates	Isolate number	Antibiotic susceptibility pattern										% of bacterial resistance to antibiotics
		SAM	**FLC**	**CM**	**CFR**	**VAN**	**GM**	**MXF**	**EM**	**TGC**	**NIT**	
Streptococcus pyogens	1	R	R	S	R	S	S	R	R	R	S	60%
Streptococcus pyogens	2	S	S	S	S	S	R	R	S	R	S	30%
Streptococcus pyogens	3	S	R	S	R	S	R	S	R	R	S	50%
Streptococcus pyogens	4	S	S	R	R	S	R	S	R	R	S	50%
Streptococcus pyogens	5	S	R	R	R	S	R	S	R	R	R	70%
Streptococcus pyogens	6	R	S	S	S	S	S	S	R	R	S	30%
Streptococcus pyogens	7	S	R	S	R	S	S	S	S	R	S	30%
Streptococcus pyogens	8	S	S	R	R	R	R	S	R	S	S	50%
Streptococcus pyogens	9	R	R	S	S	S	R	R	R	R	S	60%
Streptococcus pyogens	10	R	S	R	S	R	S	S	R	S	R	50%
Streptococcus pyogens	11	S	S	S	R	S	S	S	S	R	S	20%
Streptococcus pyogens	12	S	R	S	R	S	S	S	R	R	S	40%
Streptococcus pyogens	13	S	S	S	S	S	S	S	S	R	S	10%
Streptococcus pyogens	14	S	R	S	S	S	R	S	S	S	S	20%
Staphylococcus aureus	15	S	S	R	S	S	R	S	S	R	S	30%
Staphylococcus aureus	16	R	R	S	S	S	S	R	S	S	S	30%
Staphylococcus aureus	17	R	R	R	R	R	R	S	R	R	S	80%
Staphylococcus aureus	18	R	S	S	S	S	R	R	R	S	R	50%
Staphylococcus aureus	19	S	S	S	S	S	R	S	S	R	R	30%
Staphylococcus aureus	20	R	R	S	R	R	R	S	R	R	S	70%
Staphylococcus aureus	21	R	S	S	R	S	R	S	R	S	S	40%
Staphylococcus aureus	22	S	R	R	R	S	S	R	S	R	S	50%
Staphylococcus aureus	**23**	**R**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**S**	**50%**
Staphylococcus aureus	**24**	**R**	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**R**	**60%**
Staphylococcus aureus	**25**	**R**	**S**	**S**	**R**	**S**	**R**	**R**	**S**	**S**	**S**	**40%**
% of Sensitivity to Antibiotics		**52 %**	**52 %**	**68 %**	**44 %**	**84 %**	**36 %**	**72 %**	**40 %**	**32 %**	**80 %**	

.

Bacterial isolates Types	Isolate	Antibiotic susceptibility pattern										% of bacterial resistance to
		PTZ	CTR	CPM	ETP	NA	NOR	TOB	AMK	MNO	SXT	
Klebsialla pneumoniae	45	R	S	S	S	R	S	R	R	S	S	40%
Klebsialla pneumoniae	46	R	S	R	S	R	S	S	S	R	S	40%
Klebsialla pneumoniae	47	S	R	S	S	S	S	S	S	R	S	20%
Klebsialla pneumoniae	48	S	R	R	S	S	S	S	S	R	R	40%
Klebsialla pneumoniae	49	S	R	R	S	R	R	R	S	R	S	60%
Klebsialla oxytoca	50	R	S	R	R	R	S	S	S	S	R	50%
Klebsialla oxytoca	51	S	R	S	S	R	R	R	S	S	R	50%
Klebsialla oxytoca	52	R	R	R	S	R	S	S	S	S	R	50%
Proteus mirabilis	53	S	S	R	S	R	S	R	S	R	S	40%
Proteus mirabilis	54	S	R	R	S	S	S	R	S	R	R	50%
Proteus mirabilis	55	S	R	R	S	R	R	R	R	R	R	80%
Pseudomonas aeruginosa	56	R	S	S	S	S	S	S	R	S	S	20%
% of Sensitivity to Antibiotics		58.1 %	41.9 %	54.8 %	96.8 %	45.2 %	77.4 %	64.5 %	64.5 %	48.4 %	61.3 %	

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