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Tracing of Antibiotic-Resistant Bacteria Isolated from Semen of Iraqi Males with Primary Infertility

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Abstract

Objectives: The main objective of this study was to investigate the bacterial infections of seminal fluid in males with primary infertility. The patients were referred to and/or visiting the Urology Department in Salah Al-Deen Teaching Hospital. **Methods:** The present study was carried out from May 2017 to June 2018 and the number of the patients group was 60. The patients aged between 20 to 50 years. Semen was collected from infertile men of a couple that female failed to become pregnant after one year of regular and unprotected intercourse of marriage and submitted for seminal fluid analysis for the bacteriological analyses. The control group consisted of 50 fertile male who were selected randomly from Tikrit City during the period of this study. Semen culture was done for all the patients and the control group. **Results:** There were 30 patients who suffered from bacteriospermia and 16 of them (53.3%) were infected with gram positive organisms, while only 14 (46.7%) patients were infected with gram negative organisms. The most common organism was *Staphylococcus aureus* which was isolated from bacteriospermia. *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *S. saprophyticus*, *Proteus mirabilis* and *Niesseria gonorrhoae* were also isolated and the frequencies of isolation were 23.3% (7), 13.3% (4), 10.0% (3), 10.0% (3), 6.6% (2), and 6.6% (2) respectively. The present study showed many antibiotics were not active against gram positive bacteria isolated e.g. the resistance frequenciy to rifampcin, vancomycin and clindamycin was 48%, 44% and 40% respectively. **Conclusions:** The gram positive organisms were more prevalent than gram negative organisms in patients with bacteriospermia and the isolationfrequencies of two groups were 16 (53.3%) and 14 (46.7%) respectively. The most effective antimicrobial agents were amikacin, ciprofloxacin and levofloxacin and the least effective one was ceftazidime, whereas moxifloxacin, norfloxacin, gentamicin, azithromycin, moxifloxacin, norfloxacin, cefotaxime, and ceftriaxone were effective at different levels.

Keywords

Antibiotic Resistance, Bacteriospermia, Bacteria, Iraq

1. Introduction

Resistance to antibiotic is considered as a virulence factor for the pathogenic microorganisms to cause the infections. The first cases of antimicrobial resistance occurred in the late 1930s and in the 1940s, soon after the introduction of the first antibiotic classes, sulfonamides and penicillin. Common bacteria such as strains of *S. aureus* became resistant to these classes of antibiotics at record speed. Antimicrobial resistance is not new, but the number of resistant organisms, the geographic locations affected by drug resistance, and the breadth of resistance in single organisms are unprecedented and mounting [1].

Diseases and disease agents that were once thought to be controlled by antibiotics are returning in new leagues resistant to these therapies. However, that antimicrobial resistance is also evident in other microorganisms namely, parasites, fungi and viruses [2]. Drug-resistant strains initially appeared in hospitals, where most antibiotics were being used. Sulfonamide-resistant *Streptoccoccus pyogenes* emerged in military hospitals in the 1930s. Penicillin resistant *S. aureus* confronted London civilian hospitals very soon after the introduction of penicillin in the 1940s. Similarly, *Mycobacterium tuberculosis* with resistance to streptomycin emerged in the community soon after the discovery of this antibiotic [3]. Resistance to multiple drugs was first detected among enteric bacteria namely, *E. coli*, *Shigella* and *Salmonella* in the late 1950s to early 1960s [4]. Such strains posed severe clinical problems and cost lives, particularly in developing countries. Nevertheless, the resistance problem was perceived by some, most notably those in the industrialized world, as a curiosity of little health concern confined to gastrointestinal organisms in distant countries fueled by increasing antimicrobial use and the frequency of resistance escalated in many different bacteria, especially in the developing countries where antimicrobials were readily available without prescription. Poor sanitation conditions aided spread and small healthcare budgets prevented access to new effective but more expensive antibiotics. Since the 1980s, a re-emergence of tuberculosis has occurred that is often multidrug resistant (MDR) and enhanced by human immunodeficiency virus infection. The severity of and difficulty in treating MDR strains necessitate the use of several, sometimes six to seven different drug in susceptibility testing [5]. This study aims to assess the infection of male genital tract particularly if it happens with antibiotic-resistant bacteria and its impact on male infertility in our locality.

2. Materials and Methods

2.1. Source of Specimens

The patients are referred to and/or visiting the Urology Department in Salah Al-Deen Hospital. The number of the patients and controls were 110 (60 patients and 50 healthy fertile group) and they were recruited from May 2017 until June 2018. Semen was collected from infertile males and controls for seminal fluid analysis for the bacteriological. The control group consisted 50 persons were selected randomly from Tikrit City during the period of this study. Semen culture was done for all the patients. The inclusion criteria were as follows:

Infertile men of couples that failed to get female pregnant become pregnant after one year of regular, unprotected intercourse. Infertile man with azoospermia. Infertile man with oligozoospermia and asthenozoospermia. The control group composed of 50 subjects who were looked healthy and had comparable criteria to the patient and composed of 50 males.

2.2. Laboratory Methods

2.2.1. Culture of Urine Specimens

Media were prepared and sterilized according to the manufacturer's instruction. The prepared media used for isolation, determination of the viable count, identification and susceptibility testing were carried out after being solidified [6]. The 60 patients' group was subjected to antibiotic sensitivity testing.

2.2.2. Preparation of the Culture Medium for Antibiotic Sensitivity

Testing Antibiotic sensitivity testing of all isolates was performed on Mueller-Hinton medium by the Kerby Bauer method (1996) following the definition of the National Committee of Clinical Laboratory Standard (NCCLS, 1999). The medium was allowed to cool at 45˚C and poured into Petri dishes to about 4 mm thickness of medium. The solidified plates were incubated at 37˚C for 15 - 30 minutes to let the excess moisture to evaporate (Fisher scientific, USA) [7].

2.2.3. Inoculation and Incubation

The plates were inoculated by dipping a sterile swab into the inoculum, the excess inoculum was removed by pressing and rotating the swab firmly against the side wall of the tube above the level of fluid, then the swab was rubbed all over the surface of the medium, rotating the plate 3 times at an angle of 60 degree after each application and finally the swab passed around the edge of agar surface. The plate was left to dry at room temperature with the lid closed for few minutes. After 15 minutes of inoculation, the antibiotic discs were applied and the plates were inverted for incubation to avoid accumulation of moisture on the agar surface [7]. Maximum 5 antibiotic discs were selected and placed onto each plate using flamed forceps for application of the discs on the plate and each disc pressed down gently to ensure even contact with the medium. After overnight incubation at 37˚C the diameter of each zone including the diameter of zone inhibition was measured and recorded in mm and compared with the standard inhibition zone. For motile organisms, e.g. *Proteus* spp. the swarming haze was ignored and zones were measured at the point where growth was obviously inhibited [7].

2.2.4. Statistical Analysis

The results were expressed mean, standard deviation; statistical analysis of the results was performed using the statistical package software (SPSS), version 15.01 and Excel 2010. Pearsons correlation coefficient was used to determine the relationship between variable for all parameters. The differences were considered significant when $P < 0.05$.

3. Results

3.1. Bacteria Isolated from Semen

Semen culture was done for all the 110 subjects of study (60 patients group and 50 healthy control group) who were included in this study. Only 30 out of 60 patients group had positive semen culture as shown in **Figure 1**. There were 16/30 (53.3%) patients with bacteriospermia who were infected with gram positive organisms, while only 14/30 (46.7%) patients were infected with gram negative organisms as shown in **Figure 2**. The most common organism was *S. aureus* which was isolated from bacteriospermia. *E. coli*, *E. faecalis*, *K. pneumoniae*, *S. saprophyticus*, *P. mirabilis* and *N. gonorrhoae* were isolated and the frequencies of isolation were 23.3% (7), 13.3% (4), 10.0% (3), 10.0% (3), 6.6% (2), and 6.6% (2) respectively. Statistically, there was a highly significant difference between pyospermia (infection semen) and non-infection semen according to the distribution of the isolated bacteria (P value < 0.05).

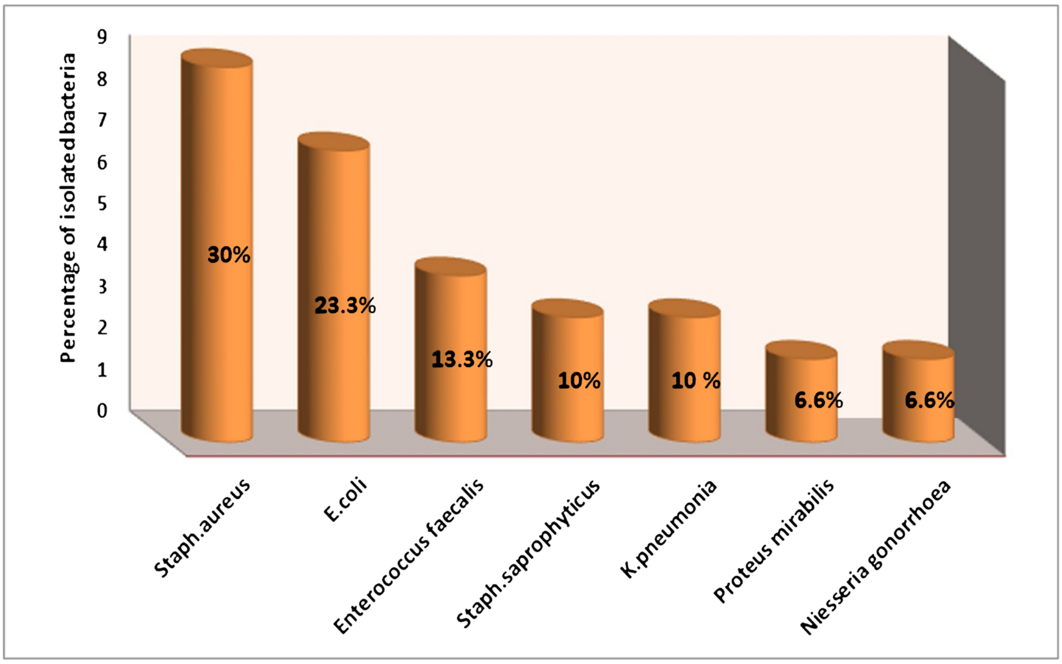

Figure 1. Percentage of isolated bacteria from semen culture of the patients.

3.2. Antimicrobial Sensitivity Testing

The antibiotic sensitivity test was carried out using disc diffusion technique and minimum inhibitory concentration for all the bacterial isolates to the most commonly antibiotic agents that used in the treatment of pyospermia as it was shown in Figure 3, Figure 4, Table 1 and Table 2.

All gram positive bacteria that isolated during present study were highly

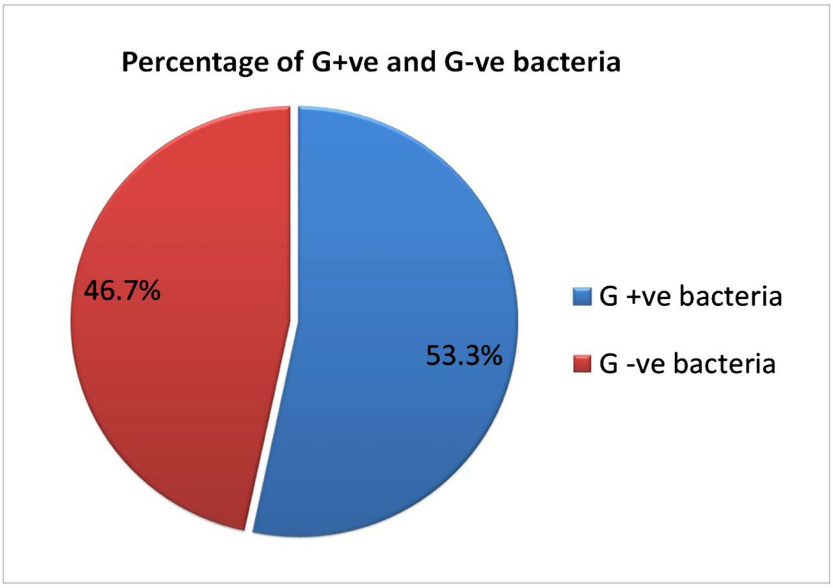

Figure 2. The rate of semen infection with gram positive and gram negative organisms.

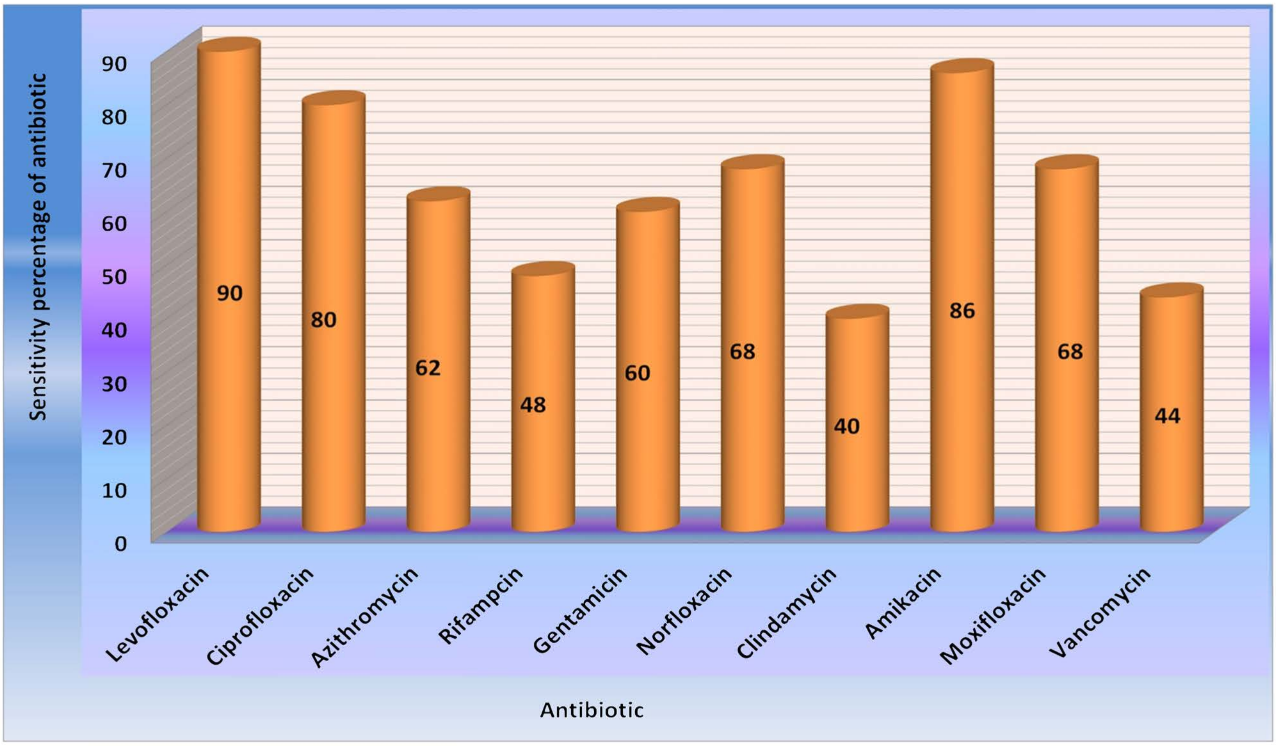

Figure 3. Total sensitivity testing for gram positive bacteria.

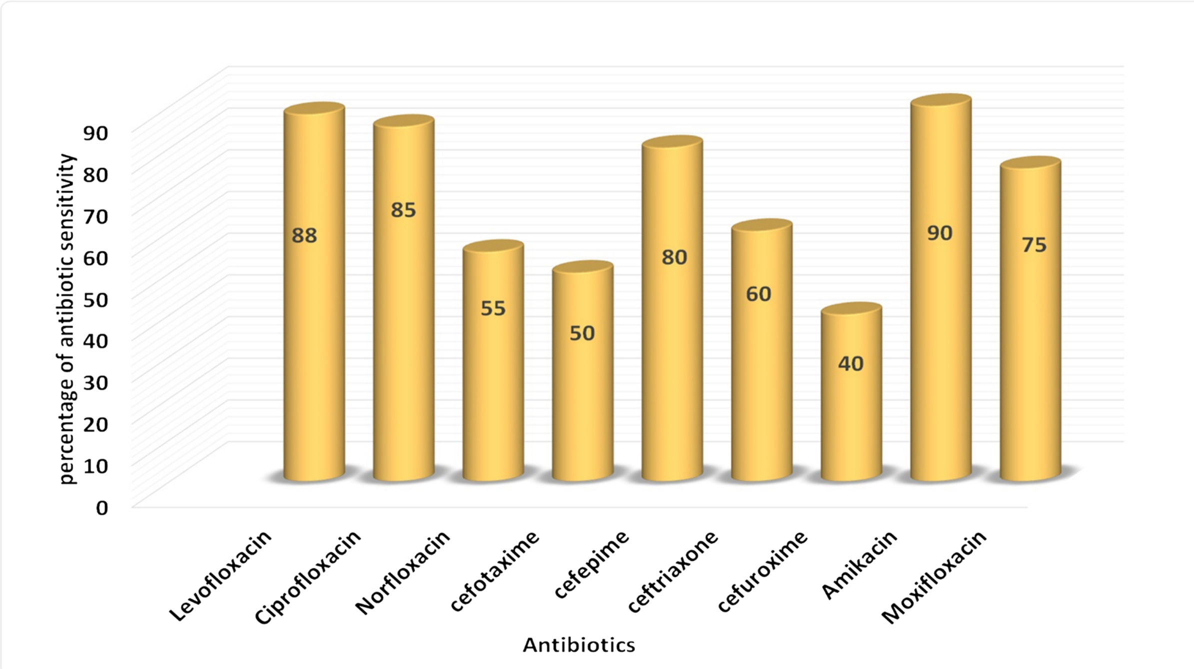

Figure 4. Total sensitivity testing for gram negative bacteria.

Table 1. Minimal Inhibitory Concentrations (μg/ml) of antibiotics that used in gram positive bacteria.

Antibiotic	Types of bacteria		
	St. aureua	*St. saprophyticus*	*En. faeculis*
Levofloxacin	1	2	2
Ciprofloxacin	1	1	1
Norfloxacin	1	1	1
moxifloxacin	1	2	2
Amikacin	4	4	4
Gentamicin	2	2	2
Vancomycin	1	2	2
Azithromycin	2	2	2
Rifampcin	2	1	1
Clindamycin	1	1	1

sensitive to the amikacin 86%, ciprofloxacin 80% and levofloxacin 90%. While moderate sensitive to moxifloxacin 68%, norfloxacin 68%, gentamicin 60% and azithromycin 62% was seen. But many antibiotics did not reveal activity against bacteria that isolated from seminal fluid as rifampcin 48%, clindamycin 40% and vancomycin 44%. Also the gram negative bacteria that isolated during present study were sensitive to the amikacin 90%, ciprofloxacin 85%, levofloxacin 88% and cefepime 80%, but were moderately sensitive to moxifloxacin 75%, norfloxacin 55%, cefotaxime 50% and ceftriaxone 60%. Other antibiotics used in the

Table 2. Minimal Inhibitory Concentrations (μg/ml) of antibiotics that used in gram positive bacteria.

Antibiotic	Types of bacteria			
	E. coli	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Niesseria gonorrhoae*
Levofloxacin	2	2	2	2
Ciprofloxacin	4	2	4	2
Norfloxacin	1	1	1	1
moxifloxacin	2	2	2	2
Amikacin	2	4	4	2
Cefepime	2	2	2	2
cefotaxime	1	2	2	2
Cefuroxime	1	1	1	2
Ceftazidime	1	1	1	2
Ceftriaxone	2	2	2	2

study such as ceftazidime 20% and cefuroxime 40% were not active against gram negative bacteria isolated during study.

4. Discussion

The results of this experiment showed that 30 (50%) specimens of subject group revealed positive bacterial culture, whereas 10 (12.9%) specimens of subject group showed no bacterial growth even after 48 hours of incubation, which may be due to the presence of another type of causative agents that might seek special technique for their detection such as viruses, *Chlamydia* or *Mycoplasma*. These results were corresponding to those results being reported by Mogra and his colleagues [8], Shefi and Turek [9]. Also, the present result was almost similar to other works reported elsewhere [10] [11]. The results in **Figure 1** were statistically analyzed which showed that there was a strong relationship between the bacteriospermia and abnormal morphology of semen in infertile male ($P < 0.05$). This result almost similar to these reported by Golshani and his colleagues [10] who stated that semen specimens of infertile men, especially those contain the high number of *Staphylococcus* spp., *E. coli* and Enterococci isolates, had high rate of non-motile and morphologically abnormal sperms. Philip and Folstad [11] confirmed that there was a significant positive effect of antibiotic treatment for the following sperm parameters: sperm volume, sperm concentration, sperm motility and sperm morphology.

The present result was conformity with Jedrzejczak *et al.* [12], who found that seminal white blood cells counts > 1×10^6/ml correlated well with bacteriospermia (more than 1000 cells/ml). A significant correlation was found between bacteriospermia and leukocytospermia at the cut-off level of $\geq 0.2 \times 10^6$ leukocytes per ml of semen specimens. The prevalence of bacteriospermia in this study was 50%, similar with higher prevalence rates of 42.9%, 51.7%, 52.5%, 65.7%, 66%, and 79% were shown by Mogra *et al.* [8], Cottell *et al.* [13], Alekwe *et al.* [14], Isaiah *et al.* [15] and Merino *et al.* [16]. While Domes *et al.* [17], have demonstrated lesser than 15% prevalence rate of bacteriospermia.

The detection of gram positive Staphylococci species from seminal fluid specimens was documented. It was found that gram positive Staphylococci species involved in the pathogenesis of chronic pelvic pain syndrome [18]. They were identified in the focal colonies adherent to the prostatic duct walls [19]. Bergman demonstrated that gram positive Staphylococci species were found in significant numbers in 43% of patients with symptoms of prostatitis [20].

Resistance of *S. aureus* and coagulase negative *Staphylococcus* to penicillin and rifampicin, clindamycin and vancomycin was presented during the present study. The results revealed the most of *S. aureus* and CoNS isolates were resistant to penicillin. Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production: an enzyme which broke down the β-lactam ring of the penicillin and its derivatives giving rise to inactivate products, cannot bind to penicillin binding proteins (PBPs) on cell wall [21] [22]. This result was in agreement with those displayed by other studies as by Al-Jebouri [23] and Al-Jebouri [24], also were consisted with those of Al-Rawi [25], Joseph and Alexander [26]. As shown in **Figure 4**, *E. coli* isolated in this study was moderately sensitive to norfloxacin and moxifloxacin, but resistance to many antibiotic ceftazidime, cefuroxime, cefotaxime, ampicilin and amoxicyllin. This resistance could be interpreted depending on the fact that many strains of *E. coli* have acquired plasmids conferring resistance to one or more than one type of antibiotics, therefore antimicrobial therapy should be guided by laboratory result test of sensitivity [27] [28]. *K. pneumoniae* (ESBL) possesses plasmid-mediated enzymes that hydrolyze oxyimino-blactam agents such as third-generation cephalosporins and aztreonam [29]. These plasmids also carry resistance genes to other antibiotics including aminoglycosides, chloramphenicol, sulfonamides, trimethoprim, and tetracycline. Thus, gram negative bacilli containing these plasmids are multidrug-resistant [30]. Furthermore, these plasmids are mobile genetic elements and can be transmitted between gram negative bacilli of different species in vivo [31]. The plasmid from *K. oxytoca* spread to *K. pneumoniae*, *E. coli*, *Enterobacter cloacae*, and *Citrobacter freundii* [32]. Resistance of 77% - 85% of *P. mirabilis* against ampicillin, cotrimoxazole, tetracycline and gentamicin was reported by Bahashwan *et al.* [33], Similar results were reported by Woo *et al.* [34]. The gram negative enteric bacterium especially *P. mirabilis* is an important cause of community- and health care-associated infections, including those involving the urinary tract, the abdominal cavity, and the bloodstream itself [35]. Like many other members of the family *Enterobacteriaceae, P. mirabilis* can harbor numerous plasmid- and integron-mediated determinants of antimicrobial resistance [36]. Multidrug-resistant (MDR) strains of *P. mirabilis* generally produce extended-spectrum β-lactamases (ESBLs) or the Amp C-type cephalosporinase and rarely carbapenemases, and their prevalence in some settings is relatively high [37] [38].

5. Conclusion

The susceptibility and resistance profile of all isolates in the current study revealed that ampicillin and amoxicillin were slightly effective while amikacin and ciprofloxacin showed the higher efficacy. There was a general increase in the resistance pattern of isolates to all the antibiotics used in this study. The current findings confirm that bacterial resistance was high and frightening problem in our country. A new generation of antibiotics is still locally working in a higher rate. The usage of antibiotics in our locality of the world needs to be under health assessment and control with more laboratory investigations to explore the intrinsic and extrinsic parameters, leading to conclude high rate of resistance occurring in the local pathogens that may be disseminated to other geographical areas.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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