

Biostatistical analysis on incidence of bacteria

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Abstract

This study investigated the incidence of bacteria and their antibiogram profile urine samples collected from students of the Northwest A&F University, Yangling, Shaanxi, China. A total of thirty (30) midstream urine samples were collected and bacteria in the samples were isolated using the spread plate method after which biochemical characterization was carried out. *Escherichia coli*, *Pseudomonas* spp, *Klebsiella* species, *Salmonella* species and *Proteus* species were the bacterial species isolated after which Kirby-Bauer disc diffusion antimicrobial assay was used to test their sensitivity against various antibiotics, such as Gentamycin, Cotrimoxazole, Ceftriaxone, Amoxycylav, Levofloxacin and Clavatin. From the study, *Proteus* spp shows zones of inhibition of (7mm for Cotrimoxazole), (5mm for Ceftriaxone), 5mm (for Gentmycin), (5mm), and 4mm (for Amoxyclav). *E. coli* 6.2 mm (Gentamycin), 3.6 mm (Levofloxacin), and 3.6 mm (Levofloxacin) zones of inhibition respectively. Gentamycin obtained 4.00mm, Levofloxacin 2.00mm and erythromycin 1.00 mm against *Pseudomonas* spp. Zones of inhibition were only observed on Clavatin (2.1 mm) for *Salmonella* species, while *Klebsiella* species was however, resistance to all the antibiotics tested. The highest sensitivity was observed in Cotrimoxazole which obtained a diameter of 7mm for *Proteus* spp., while the lowest zone was recorded for *Pseudomonas* species against Erythromycin with a diameter of 1 mm. This study revealed that most of the isolates were resistant to the antimicrobial agents the implication of antibacterial resistance as shown in the study. This study therefore recommends that proper hygiene should be observed before ingestion of food and water.

Introduction

Urinary tract infection (UTI) is the second most common infectious presentation in community medical practice. Worldwide, about 150 million people are diagnosed with UTI each year, and UTI are classified as uncomplicated or complicated [1]. UTI can occur in children as well as adults. UTIs are almost always caused by bacteria although some viruses, fungi and parasites can infect the urinary tract as well [2]. More than 85% of UTIs are caused by bacteria from intestine or vagina [2]. Bacterial infection of the lower urinary tract – the bladder and urethra are very common especially in young, sexually active women [3]. *Escherichia coli* is the commonest bacteria causing lower UTI [4]. Other Gram-negative bacteria are *Pseudomonas aeruginosa* and *Acinetobacter* spp. Gram-positive bacteria including *Enterococcus faecalis*, capnophilic coryne bacteria and lactobacilli, and staphylococci, which used to play minimal roles, have now assumed significant places as etiological agents of UTI [5]. A doctor can confirm if a patient has a urinary tract infection by testing a sample of urine. For some younger women who are at low risk of complication the doctor may not order a urine test and may diagnosed a urinary tract infection base on the description of symptoms [6]. Antibiotics are the main treatment for all UTIs. A variety of antibiotics are available and choice depend on many factor including weather the infection is complicated or uncomplicated, primary or recurrent for example, if a woman has symptoms even if bacterial count is low or normal, infection is probably present and the doctor should have considered antibiotic treatment. The following are major studies suggest may reduce the incidence of urinary tract infection [7]. A prolonged course (Six month to a year) of low-dose antibiotics (usually nitrofurantoin or Tmp/smx) is effective in reducing the frequency of UTIs in those with recurrent UTIs [6]. For post – menopausal women intra vaginal application of typical estrogen cream can present recurrent [3]. Breastfeeding can reduce the risk of UTIs in

infant [8]. Antimicrobial resistance has become an important problem worldwide (Pfaller). Bacterial resistance to antimicrobial agents has been emerging and rapidly disseminating among many nosocomial and community –acquired pathogens [9]. These organisms have wide variety of antibiotics sensitivity pattern and treatment must be guide by laboratory investigation [7]. The development of antibiotic resistance in *E. coli* has important clinical implication. The development of resistance to older antimicrobial agent such as ampicillin and trimethprim-sulfamethoxazole as well as the emerging problem of fluoroquinolone resistance may substantially limit our antibiotic choices [10].

Since first reports of transferable resistance to antimicrobial in Japan, the important of plasmids to both bacterial host and indirectly to man has been progressively appreciated [11]. At present time unfortunately, to determine phenotypic profile, conventional antimicrobial susceptibility testing method are useful for detecting resistance profile and for selecting potentially useful therapeutic agents they are insensitive tools for tracing the spread of individual strains within hospital or region. Molecular methods provide powerful tools to tract bacteria strains and contribute to the evaluation of nosocomial infection outbreak, recurrent infection and clonal dissemination of specific pathogen [12]. They are also used as a means of providing additional information to detect and evaluate the mode of dissemination of multi-drug resistance (MDR) pathogens [13].

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Key words: incidence of bacteria, antibiogram profile, pathogenic bacteria, urine specimens, students, northwest A&F University

Received: June 01, 2019; **Accepted:** June 10, 2019; **Published:** June 13, 2019

The molecule characterization of microorganisms is frequently used by physicians, microbiologist, and epidemiologist to provide evidence of genetic relatedness as an aid in the epidemiological investigation of infectious diseases [12]. The needs for determining the relatedness of organisms may arise during an outbreak investigation in which a cluster of infection cause by organisms of the same species showing similar antimicrobial resistance profile and in order to determine clonal spread within a microenvironment, and to determine the source of infection [12].

It has also been established that intervention in the normal physiological function of bladder emptying is a major factor in the development of UTIs [3]. Patient with suspected cases of UTIs are known to undergo self-medication which leads to resistance of bacteria to antibiotic. It is against this background that this research was designed in order to identify the most common causes of this problem and ascertain the pattern of its sensitivity to antibiotics. Pathogenic bacteria have been implicated as the etiological agent of so many life-threatening infections and it is also known for its remarkable ability to develop antimicrobial resistance [14]. Despite the foregoing and the continuous observation of improved pathogenicity of bacteria and its associated resistance to antimicrobials especially in regard to urinary tract infections (UTIs), only few or no documented report on its incidence and antimicrobial resistance via bacteria production. This study therefore aimed at checkmating the incidence of bacteria and antibiogram profile of pathogenic bacteria in urine and to proffer promising recommendation based on the findings [15]. This study, therefore, ascertained the incidence of bacteria and antibiogram profile of pathogenic bacteria in urine samples from students of Northwest A&F University.

Materials and method

Freshly voided mid-stream urine samples were received between June 2018 and July 2018 and a total of 30 samples were collected from both male and female students of Northwest A&F University, China.

This study was carried out at the College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, China. Samples were collected from hospitals in Northwest A&F University.

Sample size

A total of 30 midstream urine samples were collected from students of College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, China. The samples were collected in sterile centrifuge tube and immediately transported to the laboratory for analysis.

Isolated bacterial pathogens

The urine samples were screened for the presence of urine pathogens. 0.1ml of each urine sample was inoculated on Cysteine Lactose Electron Deficient (CLED) agar using spread plate technique, allowed to stand for five (5) minutes and incubated inverted for 24 hours at 37 °C. Colonies that exhibited pigmentation were taken sub-cultured to obtain pure culturing on Nutrient agar (N.A).

Morphological identification and biochemical tests

Morphological identification of the isolates and all biochemical tests of the presumptive isolates were done according to standard methods.

Antimicrobial susceptibility test

Antimicrobial sensitivity was tested for each isolated organism using the disk diffusion method of each isolated organism using

the disk diffusion method of Kirby-Bauer as described by the National Committee for Clinical Laboratory Standard. The multi disc contains the following antibiotic for gram negative organisms and they are Ampicillin (Amp), Gentamycin (GEN), Nitrofurantoin (NIT), Contrimazole(COT), Ofloxacin(OFX), Cefuroxime(CER), Tetracycline(TET) and Cephalexin(CEPH).

Result and discussion

Out of the 30 samples collected, 12 were males and 18 were females, 11 males were positive 17 females were positive also, the percentage of the positive cases of males was 39.3 and 60.7 for females as presented in table one below.

Table 1 below shows the distribution of cases and their prevalence between male and female students of Northwest A&F University, were a total of 30 samples was collected, the % Positive cases of male students was 39.3%, and % Positive cases of female students was 60.7% as shown in table 1 below.

Table 2 below shows bacteria distribution of positive cases without prevalence rate. The result obtained revealed that *E.coli* had the highest prevalence rate of 32.4% followed by *proteus* and *salmonella* specie with prevalence rate of 8.8%, *klebsiella* and *Pseudomonas* species have a prevalence rate of 11.8% while other Gram negative bacteria had the prevalence rate of 26.4% as shown in Table 2 below.

Table 3 below shows the antimicrobial sensitivity assay of the bacterial isolates. The result shows that *E. coli* has 6.2 mm (Gentamycin), 3.6 mm (Levofloxacin) zone of inhibition, *klebsiella spp.* was resistance to all antibiotics tested, *Pseudomonas spp.* shows 4mm zones of inhibition for Gentamycin, Levofloxacin 2 mm and erythromycin 1 mm against *Pseudomonas spp.* While *proteus spp.* obtained zones of inhibition of (7mm for Cotrimoxazole), (5mm for Ceftriaxone), 5mm (for Gentmycin), (5mm), and 4mm (for Amoxyclav). For *Salmonella spp.* zone of inhibition was only observed on Clavatin (2.1mm) as shown in the table 3 below.

A total of 30 samples collected from Northwest A&F University's students were investigated. Out of the 30 samples, 18 samples were from females while 12 samples were from males within the age range of 18-25 years. Out of the 30 samples collected, all the 30 samples were positive, 18 were female while 12 were males. The 12 samples collected from males were all positive with prevalence rate of 39.3% while 18 samples from females were also positive with prevalence rate of 60.7% as shown in Table 1. Therefore, the prevalence rate of positive cases for male and females were 39.3% and 60.7% respectively.

Table 1. Sex distribution of cases and their prevalence rate (18-25yrs)

Sex	Total case	Positive cases	% Positive cases
Male	12	11	39.3
Female	18	17	60.7
Total	30	28	100

Table 2. Bacteria distribution of Positive cases without prevalence rate

Bacteria isolate	Positive cases	% Positive cases
<i>E. coli</i>	11	32.4
<i>Klebsiella spp</i>	3	8.8
<i>Pseudomonas spp</i>	3	8.8
<i>Proteus spp</i>	4	11.8
<i>Salmonella spp</i>	4	11.8
Others (Gram negatives)	9	26.4
Total	34	100

Table 3. Antimicrobial sensitivity assay of the bacterial isolates

ISOLATES	CIT	COT	AMC	CTR	OF	EM	GEN	LEV
<i>E. coli</i>	0mm	0mm	0.00mm	0mm	0.00mm	2mm	6.2mm	3.6mm
<i>Klebsiellaspp</i>	0mm	0mm	0.00mm	0mm	0.00mm	0.00mm	0mm	0.00mm
<i>Pseudo spp</i>	0mm	0mm	0.00mm	0mm	0mm	0.00mm	4mm	2.00mm
<i>Proteus spp</i>	0mm	7mm	4.00mm	5mm	0mm	.00mm	5.00mm	0.00mm
<i>Salmonella spp</i>	0mm	0mm	2.11mm	0.00mm	0mm	0mm	0mm	0.00mm

Key: (Gram +ve) = Gram positive, CIT = Galcipro TN (Ciprofloxacin/Tinidazole), COT = Primipex (COTRIMOZAXOLE), AMC = Clavatin (Amoxycylav), CTR = Cefax (Ceftriaxone) OF= Ofloxacin, EM = Erythromycin, GEN=Gentamycin LEV=Levofloxacin.

E. coli had the highest prevalence rate of 32.4% followed by *proteus* and *salmonella* specie they had the prevalence rate of 8.8% so also *klebsiella* and *pseudomonas* species they had the prevalence rate of 11.8% while other gram positive had the Prevalence rate of 26.4% as shown in table 2 above.

It was also observed that Gentamycin, Nitrofurantoin and Ofloxacin were the most sensitive antibiotics in the study while tetracycline Cephalixin, Ampicillin and Cotrimozole gave poor sensitivity or resistance. As shown in table 3 from this measure above. Gentamycin, Nitrofurantoin and Ofloxacin proved the best antibiotics against Gram negative bacteria.

The overall prevalence of UTI in this study was 91.2% and females were significantly more affected than males. The present study conforms with the works of [16,17] carried out in different parts of the world indicates higher incident among females than males, this may be attributed to the fact that females pass short urethra [17].

Also the spread of normal flora in fecal materials from the anus to the vagina from where the bladder could be infected as a result of poor anal cleaning could be responsible for the observed result in female urine sample [18].

The prevalence of UTI in Northwest A&F University which is found to be 91.2% is quite alarming. This calls for caution among the female students in Northwest A&F University. The high rate may be due to increase in female with poor hygienic practice and also indiscriminate sexual behavior among the female students [19].

E. coli was the commonest organism isolated; this is in conformity with the previous work. The factors contributing to those resistances may be due to indiscriminate abuse of antibiotic by students. Other factors may include poor quality of drugs, poor storage and exposed drug [20] etc. the reduction of antibiotics prescription and dispensation have been associated with reduced antibiotic resistance [21].

Conclusion and recommendation

This study has revealed that UTI among female students is a very difficult health problem which must be properly addressed. This has also revealed that the most causative organisms of UTI in this university, community among the female students are the gram-positive organisms which were shown to be sensitive to the following drugs; Gentamycin, levofloxacin and cotrimozaxole. It could be suggested that in the face of clear UTI symptoms and in the absence of physician or clinician of these three-drug abuse (Gentamycin, levofloxacin and cotrimozaxole) could be procured and used with an experienced doctor is seen for confirmation.

It is therefore recommended that antibiotics therapy should be used only after a thorough culture and antibiotics sensitivity test have been carried out to avoid the emergence of drugs resistance among bacteria. It should be recommended that personnel hygiene and education on method of prevention and transmission of UTIs should be strengthened. Also, since the hospital environment is a sort of

collection agency for many pathogenic organisms by virtue of the many serious ill patients who passes through it, it is therefore important for the hospital management to do everything possible to minimize the spread of these organisms to other patients.

Acknowledgement

The authors will like to thank everyone who has assisted in the successful outcome of this work.

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