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Isolation and Identification of Urinary Tract Infectious Bacteria and Exploring their Anti-drug Potential against Some Common Antibiotics

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Abstract

Urinary tract infection caused by bacteria leads to inflammation and over growth of uropathogens and prevalence of infection for both genders, but women is more vulnerable especially at the sexually active ages. Nine isolates from sixteen patients were microscopically tested, characterized, identified using different media and biochemical tests. The highest rate of isolated bacteria were *Escherichia coli* and *Staphylococcus aureus* (23.52%), followed by *Staphylococcus saprophyticus*, *Entrococcus faecalis* (17.64% and 8.82), respectively and *Entrobacter aerogenes*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were (5.88%), only 2.94% of bacteria was detected as *Proteus miralilis*. Effect of different antibiotics was reported, maximum effect showed by Gentamycin and Chloramphenicol (80% and 70%), respectively. Contrastingly, levofloxacin 50%, Amikacin and Nitrofurantoin 40%, Ceftriaxone and Amoxicillin 30%, Cefixime 10%. In conclusion, unsuitable medication prior to urine culturing causes to increase prevalence of gram positive bacteria as much as gram negatives and developing multidrug resistance.

Keywords: Urinary tract infection; Uropathogenic bacteria; Antibiotic; Resistance; Agar well diffusion

Introduction

Urinary Tract Infection is classified as the most common and occurring nosocomial bacterial infection in human populations around the world [1-3]. UTI is a condition caused by pathogenic invasion of the epithelium, which lines the urinary tract from the minor calyx to prostatic urethra. The proliferation of bacteria in the urothelium can be asymptomatic or symptomatic, which causes inflammatory response and symptomatic case characterized by a wide range of symptoms including, fever, lethargy, anorexia and vomiting [4-9]. However, both genders are susceptible to this type of infection, but women are more, as their reproductive anatomy and physiology are more sensitive. Half of all women by 32 years age had experienced at least an infection history [7,10].

Normally, urinary tract urine mostly dominated by *E. coli* 75%-80%, followed by *S. saprophyticus* 10-15% [11-15]. While, Anatomy or physiological factors cause abnormality of urinary tract and lead to localize infectious bacteria, such as different species of Klebsiella, Proteus, Enterobacter, Enterococcus, Staphylococcus and *Pseudomonas aeruginosa.* Those bacteria are more common in most of the cases, and infrequently cause to uncomplicated cystitis and pyelonephritis [11,12,16]. Furthermore, pathogenesis of Urinary tract is more complicated and influenced by other factors, such as vaginal ecosystem especially *Lactobacillus* spp., intestinal population, genetic and behavioral factors, virulence properties of uropathogens and host defense factors [17-19]. The presence of factors will increase opportunity for uropathogens to colonize and invade urothelium [20-22].

Treating urinary tract must be done by the correct diagnosis of symptomatic patients and adequate antimicrobial therapy is taken, with correct dose and route of administration. Intestinal mucosal protection as a mechanical barrier proposed to reduce uropathogen adherence to urothelium. Bacterial proliferation and decrease of the load of UPEC in the intestinal lumen and in the fecal material as non-antibiotic approach therapy [20,23].

As UTI is one of the most prevalent problem among people of all ages and both genders and because the infection mostly associated with gram negative uropathogenic *E. coli* (UPEC) [13]. For these reasons, the antibiotics which are prescribed by physician mostly effective against gram negative uropathogen. Actually, in most cases of UTI associated with bacterial infection many species of gram positive bacterium are detected as infectious agent. In order to maintaining the level of bacterial normal flora at the appropriate rate and prevention from growth of antibacterial resistance pathogen due to the usage of unnecessary and impracticable antibiotic [24].

Our aim is to isolate, characterize and identify the uropathogens in our endemic region and determine the susceptibility of each isolated organism against several selected antibiotics, which are commonly consumed by patients against bacterial infection in urinary tract. The mean point of this study is to perform the consciousness for using the urine culture sensitivity for each patient and detecting the potential of antibiotics qualitatively and quantitatively prior to use any course of antibiotic or anti-inflammation therapy.

Materials and Methods

Sample collection

Patients were asked to clean their external genitalia with disinfectant and collect midstream urine in sterilized cap. Samples were kept in ice bag and directly transported to microbiological laboratory.

Physical examination

(pH, color, volume, appearance) parameters of collected urine specimens were analyzed.

Urine culturing

Urine samples were cultured on Nutrient agar, blood agar and

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MacConkey agar medium and incubated over night at 37°C. Significant growth was evaluated as ≥ 10^5 colony-forming units CFU/mL of midstream urine.

Characterization and identification

Culture characteristics: Each of the color, size, elevation, margins and texture of colonies were screened. The morphological different colonies on MacConkey agar, nutrient agar and blood agar were sub-cultured into nutrient agar medium, in order to purify the isolated bacteria from each patient urine specimen.

Microscopically examination: Pure isolates were examined microscopically, on the base of their cell wall composition and presence of capsule.

Microbiological analysis: According to gram staining technique, isolates were cultured on numerous selective and differential media to find out their color, colony morphology and ability of fermentation.

Prepared media

Several types of media were used to grow up as well as characterize obtained isolated, such as; Nutrient agar, Mac Conky agar, Blood agar, Pepton water, Simmon citrate agar, EMB, MSA, MHA, Casein agar, TSI, Semi-solid nutrient agar.

All the above media were autoclaved at 121°C for 15 min.

Biochemical test

Selected colonies were identified and differentiate according to the culture characteristics, microscopical examination and microbiological analysis were tested biochemically for further confirmation of isolated bacteria, such as; TSI, Catalase, Oxidase, Indole production test, MR test, VP test, Citrate utilization test, Motility test and Casein hydrolysis.

Antimicrobial sensitivity testing (Kirby-Bauer method)

The susceptibility of isolates to antibiotics were demonstrated by using nine specific antibiotics, including prescribed antibiotics that have been given by physician (Gentamycin, Penicillin, Amikacin, Chloramphenicol, Cefixime, Ceftriaxone, Amoxicillin, Nitrofurantoin, Levofloxacin).

Isolates were inoculated in peptone water and incubated in 37°C, 18-24 h. Next, they were re-cultured in broth and their turbidity compared to 0.5 Mcfarland standard solutions. More ever, new cultures were plated on Mueller-Hinton agar by swabbing. After drying for about 5-10 min, Plates were incubated for about 10-15 min at 37°C. Furthermore, interested antibiotic discs were adjusted on cultured plates using sterile forceps and incubated as inverted for 24 h at 37°C.

Results and Discussion

Urine sample of sixteen patients were collected from Ashti Hospital, Soran city, Kurdistan. Microscopically, they were examined and physical properties recorded as a primitive stage of detecting inflammation, due to the presence of RBCs, crystal, epithelial cells, color, reaction and appearance as shown in Table 1. Samples were cultured and thirty four different bacteria obtained from nine different species. For this reason, all the colony characteristics were recorded to more recognize isolates and differentiation between them on various types of media (Table 2), because monitoring bacteria on media is an initial achievement to identify bacteria on the basis of colony appearance [25]. Furthermore, among nine isolated bacteria only three were gram positive and cocci shape, the remained gram negative and bacilli shape.

Patient code	Age	Gender	Color	Appearance	pH
1	25	Female	white	Clear	Acidic
2	18	Female	Pale yellow	Turbid	Acidic
3	32	Female	White	Clear	Acidic
4	22	Male	Yellow	Clear	Acidic
5	30	Female	Yellow	Clear	Acidic
6	35	Male	Yellow	Turbid	Acidic
7	32	Female	Pale yellow	Clear	Acidic
8	40	Female	Red	Turbid	Acidic
9	65	Male	Yellow	Turbid	Acidic
10	27	Male	Yellow	Clear	Acidic
11	38	Male	Pale Yellow	Clear	Acidic
12	25	Female	Yellow	Turbid	Acidic
13	27	Female	White	Clear	Acidic
14	48	Male	Yellow	Clear	Acidic
15	21	Female	Yellow	Clear	Acidic
16	23	Female	Yellow	Turbid	Acidic

Table 1: Physical parameters of urine samples.

Patient No.	Isolates	Number of uropathogen present
1	*Escherichia coli* *Staphylococcus saprophyticus*	2
2	*Escherichia coli* *Staphylococcus aureus*	2
3	*Escherichia coli*	1
4	*Staphylococcus saprophyticus* *Staphylococcus aureus*	2
5	*Klebsiella pneumonia* *Enterobacter aerogenes* *Staphylococcus aureus* *Staphylococcus saprophyticus* *Proteus mirabilis*	5
6	*Staphylococcus aureus*	1
7	*Enterobacter aerogenes*	1
8	*Staphylococcus aureus* *Staphylococcus saprophyticus* *Proteus vulgaris*	3
9	*Staphylococcus saprophyticus*	1
10	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Escherichia coli*	3
11	*Enterococcus faecalis*	1
12	*Escherichia coli* *Staphylococcus aureus*	2
13	*Pseudomonas aeruginosa* *Kliebsiella pneumonia* *Escherichia coli* *Proteus mirabilis*	4
14	*Staphylococcus aureus* *Escherichia coli* *Enterococcus faecalis*	3
15	*Staphylococcus saprophyticus*	1
16	*Staphylococcus aureus* *Enterococcus faecalis* *Escherichia coli*	2

Table 2: Isolated bacteria in urine sample.

Various types of specific media were prepared to cultivate and characterize isolates, such as; Blood agar, Nutrient agar for all isolates. EMB and MacConky agar for gram negative bacteria, while MSA used as a selective and differential medium of *S. aureus* (Table 3).

Then, several biochemical tests were performed for gram negatives, such as IMViC test and TSI as the most special tests to identify them. Regarding present or absent of catalase and oxidase enzyme, there were only *E. faecalis* (-) and *P. aeroginosa* (+) was respectively. Motility test was applied to all isolated bacteria, all gram negatives motile excluding *K. pneumonia*, while no gram positive motile. Casein was used to differentiate *Enterococcus faecalis* and *E. coli* from other gram negatives, which indicated their ability to casein hydrolysis (Table 4). *S. saprophyticus* was resistant to Novobiocin, which is the only promised antibiotic that allows all species of *Staphylococcus* to grow except *S. saprophyticus* [26].

As a result, nine species were identified from sixteen patients, the

Media	Uropathogen	Colony morphology
MacConkey agar	*Escherichia coli*	Lactose-fermenting 'shiny, rose pink
	Klebsiella pneumonia	Pink, colony, large 'glistening and mucoid
	Pseudomonas aeruginosa	Flat, blue-green diffusible pigment, feathery
	Proteus mirabilis	Lactose-non fermenting 'colourless colony
	Proteus vulgaris	Lactose-non fermenting 'colorless colony
	Enterobacter aerogenes	Lactose fermenter, light pink, unbonate small
Eosin methylene blue agar	*Escherichia coli*	Green metallic sheen colonies
	Klebsiella pneumonia	Large, pink to purple colonies
	Pseudomonas aeruginosa	Diffusible , purple 'rough
	Enterobacter aerogenes	Pink, colony without metalic green sheen
	Proteus mirabilis	Irregular spreadable, small, colorless colony
Mannitol salt agar	*Proteus vulgaris*	Small color less colony
	Staphylococcus aureus	Yellow colony, mannitol fermenter
	Staphylococcus saprophyticus	Fermenter mannitol non-color less to white
Blood agar	*Enterococcus faecalis*	White color weak mannitol fermenter
	Escherichia coli	Shiny, opaque, creamy .B-hemolytic
	Klebsiella pneumonia	y-hemolysis, hemolysis, grey color
	Pseudomonas aeruginosa	B-hemolysis Grayishcolonies, oblique lighting
	Enterobacter aerogenes	White circle colony of Y-hemolysis
	Proteus mirabilis	Swarming spread film growth white to grey
	Proteus vulgaris	Non swarming grey colony
	Staphylococcus aureus	Small white to creamy, B-hemolysis
	Staphylococcus saprophyticus	White circle colony of Y-hemolysis
	Enterococcus faecalis	Small smooth colony, Y-hemolysis
Nutrient agar	*Escherichia coli*	Circle, convex, small colonies
	Klebsiella pneumonia	Circle, convex, small mucoid colonies
	Pseudomonas aeruginosa	Large, opaque, produce diffusible pigment
	Enterobacter aerogenes	Small, creamy convex, smooth
	Proteus mirabilis	Spreadable, swarming white to creamy colony
	Proteus vulgaris	Non swarming circular smooth entire, opaque
	Staphylococcus saprophyticus	White to pink circular*convex
	Enterococcus faecalis	Small, entire, circular, opaque, white convex
	Staphylococcus aureus	Entire, white pinhead (punctiform)convex

Table 3: Isolated bacteria on different media.

BIOCHEMICAL TESTS	*E. faecalis*	*E. coli*	*K. pneumonia*	*S. aureus*	*P. vulgaris*	*S. saprophyticus*	*P. mirabilis*	*P. aeruginosa*	*E. aerogenes*
Gram stain	+	-	-	+	-	+	-	-	-
Shape	Cocci	Rod	Rod	Cocci	Rod	Cocci	Rod	Rod	Rod
Capsule stain	-	-	+	-	-	-	-	-	-
TSI	A/A	A/A,G	A/A,G	A/A	A/A,G,H_2S	A/A	K/A,G,H_2S	K/K	K/A,G
Catalase	-	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	+	-
Indole production test		+	-		+		-	-	-
MR test		+	-		+		+	-	-
VP test		-	-		-		-	-	+
Citrate utilization test		-	+		-		+	+	+
Motility test	-	+	-	-	+	-	+	+	-
Casein hydrolysis	+	+	-	-	+	-	-	-	-

Table 4: Biochemical test for isolated bacteria.

Antibiotic (mcg)	*E. coli*		*K. pneumonia*		*P. aeruginosa*		*E. aerugenes*		*P. mirabilis*		*P. vulgaris*		*E. faecalis*		*S. aureus*		*S. saprophyticus*		% OF
	S/R	mm	S/R	mm	S/R	mm	S/R	mm	S/R	mm	S/R	mm	S/R	mm	S/R	mm	S/R	mm	sensitivity
Levofloxacin (L) (5 mcg/10 mL)	S	24	R	-	S	14	R	-	S	21	R	-	S	28	S	18	R	-	50
Amoxicillin (AMX) (25)	S	22	R	-	S	23	R	-	R	-	R	-	S	17	R	-	R	-	30
Gentamycin (G) (8000 mcg/2 mL)	S	25	S	27	S	23	S	26	S	27	R	-	S	26	S	23	S	17	80
chloramphenicol 30	S	25	m	13	S	12	R	-	S	16	S	20	S	25	S	25	S	28	70
Amikacin (AK) (10)	S	17	R	13	S	14	R	13	R	13	S	19	S	20	R	14	R	10	40
Nitrofurantoin (N) (50 mcg/mL)	S	23	S	18	S	11	R	-	R	-	R	-	S	15	M	15	R	-	40
cefixime (CFM) (5)	R	14	R	-	R	-	R	-	R	-	R	-	R	-	S	14	R	-	10
Ceftriaxone (CRO) (30 mcg)	S	26	R	-	S	23	R	-	R	11	R	-	R	20	S	27	R	-	30

S: Sensitive; R: Resistance

Table 5: Antibiotic sensitivity test Kerby-Bauer method.

highest rate of bacteria were *E. coli* and *S. aureus* (23.52%), followed by *S. saprophyticus*, *Enterococcus faeculis* (17.64 % and 8.82), respectively. *E. aeroginosa*, *K. pneumonia*, *P. vulgaris* and *P. aeroginosa* (5.88 %), and only 2.94% of bacteria was detected to be *P. miralilis*. According to the highest rate of infections due to *E. coli* and maximum number of isolates is *E. coli*. However, *S. aureus* can be seen in urinary tract but less than *E. coli*, but in this study this rate was as much as *E. coli* high, may be due to contamination of urine during urinate, which is caused by bacteria on skin, or no use of disinfectants to remove microorganisms on skin in this area [19,27]. Sensitivity and resistance of isolates for various antibiotics were reported in Table 5.

Gentamycin was 80% effective, followed by Chloramphenicol 70% and Levoxacin 50%, Amikacin, Nitrofurantoin 40% and both Ceftriaxone and Amoxicillin 30%, less effectiveness showed by Cefixime 10%. The obtained data of this study were represented that most of the people who are infected by UTI, belong to the age group of 25-35 years [28,29].

Conclusion

Nine isolate of bacteria were identified from sixteen patients. Their sensitivity to nine antibiotics was performed and the activity of antibiotics for inhibiting bacterial growth was at different levels, According to their ability. We concluded that people at age group of 25-35 highly susceptible to got highest number of uropathogens and most of the gram positive bacteria were resist to antibiotics that specified to UTI and most commonly given by consultants directly without culturing.

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