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Biochemical characterization and antimicrobial susceptibility trends of *Proteus mirabilis* isolated from patients suspected with urinary tract infections attending Sickbay Hospital, Zaria, Kaduna, Nigeria

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ABSTRACT

The research study on the biochemical characterization and antibiogram pattern of Proteus mirabilis isolated from patient suspected with urinary tract infections attending Sickbay hospital Ahmadu Bello University, Zaria was carried out. A total of 100 urine samples were collected from patients suspected with urinary tract infections, and screened for Proteus mirabilis using standard microbiological methods, out of which 20(20%) tested positive. The result shows that Streptomycin 20(100%), Erythromycin 17(85%), Ciprofloxacin 14(70%), and Sparfloxacin 15(75%) have the highest activity against the isolated Proteus mirabilis. While Proteus mirabilis recorded high resistance to Amoxicillin 20(100%), Tetracycline 19(95%), and Zinnacef 16(80%). The isolated Proteus mirabilis showed multiple drug resistance ability to the antibiotics used. It is concluded that all the antimicrobial agents that were found to be active against the test organisms can be used as the first-line drugs of choice for the treatment of urinary tract infections caused by Proteus mirabilis. Those antimicrobial agents that showed relative inactivity against the isolated Proteus mirabilis should be discouraged in the treatment of infections caused by the bacterium.

Keywords: Antimicrobial, Biochemical Characterisation, Isolation, Proteus mirabilis, Urinary Tract Infection

INTRODUCTION

Among long-term care residents, urinary tract infections (UTIs) are the second most common infection responsible for hospital admission, second only to pneumonia. UTIs can result in sepsis if not recognised and treated rapidly. Failure to treat or delay in treatment can result in a mortality rate of 20%-50%. Other factors that increase infection rates include; sex, duration of catheterisation, underlying illness, faulty catheter care and lack of systemic antibiotic therapy. Infections occur either by migration of bacteria up to the catheter along the mucosal sheath or by migration up to the catheter lumen from infected urine [1].

Despite recent advances in the diagnosis and administration of effective antibiotics in the treatment of urinary tract infections, yet many microorganisms such as *Proteus mirabilis* are becoming resistant to the commonly used antibiotics used in the treatment of urinary tract infections by virtue of the bacterial ability to produce *beta-lactamase* and acquisition of resistant plasmids. Therefore, periodic determination of the bacterial antibiogram pattern is imperative to detect any development of resistance against previously active drugs. The research went a long way to determine the first line drug of choice to be used in the treatment of diseases caused by *Proteus mirabilis* [1].

The main aim of this research work is to isolate, biochemically characterize and subject the *Proteus mirabilis* isolated from patients attending Sickbay Hospital, Zaria to antibiotics susceptibility test. The research is based on the biochemical characterization and antibiogram of *Proteus mirabilis* isolated from patients suspect with urinary tract infections. Molecular and serological characterizations of the isolates are beyond the scope of this study.

Proteus mirabilis was first discovered by a German scientist named Gustav Hauser [2]. Hauser named this genus *Proteus*, after the character in Homer's the odyssey that was good at changing shape and evading being questioned [2], a name that seems apt given this organism uncanny ability to avoid the host's immune system. *P. mirabilis* is a gram-negative, rod-shape bacterium that can be found as part of the micro-flora in human intestine. This organism is not usually a pathogen, but does become a problem when it comes into the other parts of the body. It is one of the species within the *Proteus* genus responsible for causing urinary tract infections in thousands of people each year in hospitals. *Proteus mirabilis* is the common pathogenic bacteria associated with urinary infections that occur in hospital settings and ninety per cent of bacterial UTIs involve *Proteus* infections [3]. Its genome codes for at least 10 adhesion factors making this organism extremely sticky and motile. *Proteus mirabilis* tests indole-negative, and can be easily identifiable in a blood by forming a concentric rings of its swarming movement [2] and [4].

Proteus mirabilis causes infections in human only when the bacteria leave the intestinal tract. They are found in urinary tract infections and produce bacteremia, pneumonia and focal lesion in debilitated patients or those receiving intravenous infusion. *Proteus mirabilis* causes urinary tract infections and occasionally other infections. *Proteus mirabilis* and other non-fastidious gram-negative rods are mostly encountered significant isolates in many clinical specimens in all part of the world [5].

Proteus mirabilis is normally found in the human intestine with other organisms composing a highly complex in micro-flora. They also inhabit other external environments, and are specially prevented in hospitals and healthcare facilities by using disinfectants. Interestingly, *P. mirabilis* have been known to inhabit skin & mucous of both patients and personnel working in these environments which may be the primary vectors for pathogenicity [6].

Urinary tract infections (UTIs) are very painful and can become lethal if the infection spread to other system in the body. After pneumonia, urinary tract infections are the most common problem in long-term hospital patients. These infections are becoming more difficult to treat because forty-eight per cent *Proteus mirabilis* strains are resistant to amoxicillin, penicillin, flouroquinolones and other broad range activity antibiotics. The pH of urine is usually neutral or slightly acidic, but when a patient wear a catheter for extended period of time crystalline deposited from the urine form a crust around the catheter and obstruct urine from moving through the urethra. The encrusted crystals on the catheter give *P. mirabilis* the opportunity to colonise in large numbers and to hydrolyse the urea, thus increasing the environmental pH through the production or ammonia [7] [8].

Metabolically, *Proteus mirabilis* is involved in urease production which is then converted to ammonia. This may be one of the reasons the pathogen is so successful in colonizing the urinary tract and causes infection in humans [9].
Mobility in *Proteus mirabilis* is highly complex as they engage in several different kinds of movement depending on the specific environment they are inhabiting. Most of these movements are directly tied to the differential expression of flagella and other factors. When in liquid environment, normally movement is facilitated by swarming. However, in more viscous and solid environments, *P. mirabilis* have the ability to differentiate in elongated, multinucleated, highly flagellated cells, which then allow them to move together over solid surfaces at a very high rate [10].

The activity, known as swarming, is a primary factor in success of *Proteus mirabilis* in causing complicated urinary tract infection and other more serious bladder and kidney infections. Urinary tract infections (UTIs) due to *P. mirabilis* are usually a secondary result of long-term catheterization in those who have the urinary structural abnormalities [11].

The bacteria's ability to swarm over surfaces allows them to ascend up the urethra, eventually invading the bladder and kidney, and lead to more complicated problems, such as bladder/kidney stones. In rare cases, *P. mirabilis* is able to enter the bloodstream inducing a systemic inflammation response syndrome (SIRS), which has a mortality rate of 20%-50% [12].

Proteus mirabilis is a common pathogen responsible for complicated urinary tract infection (UTIs) that sometimes it causes bacteremia. Most cases of *P. mirabilis* bacteremia originated from UTI, however, the risk factor for bacteremia and mortality rate from *P. mirabilis* UTI have not been determined. *Proteus mirabilis* are resistant to some antibiotics and it has the ability to form bio-films. Therefore, the periodic determination of antibiotic susceptibility pattern of the bacterium is imperative in order to detect an emergence of resistance to previous active drugs [13].

Under suitable condition, *Proteus mirabilis* is an opportunistic pathogen which invades and cause septic lesion at other sites of the body. They have been isolated from infection of wounds, burns respiratory tract, eyes, ears and throat [14].

Proteus mirabilis causes urinary tract infection primarily through indwelling catheters. Usually the urinary tract system can wash out the microbes before they accumulate, but the catheter prevents this from happening. *Proteus mirabilis* can then adhere to the inside and outsides of the catheter, forming bio-film communities. Once established, these microbes pass through the urethra via swarming motility to the bladder. *P. mirabilis* binds to bladder epithelial cells where it eventually colonized [6] [8].

Proteus mirabilis infection can lead to the production of kidney and bladder stones. The bacteria colonized the stones as they form, making them less accessible to antibiotic attack [15].

There are four possible mechanisms by which *P. mirabilis* can used to evade the host defences. The first is the production of an IgA-degrading protease which functions to cleave the secretory immunoglobulin A (IgA). IgA is released by the host in an initial response to infection [16].

The second immune system evasion mechanism is through three unique flagellin genes, which have been shown to recombine and form novel flagella capable of tricking the host's defence [17].

The third is through expression of MR/P fimbriae. They go through the process called phase variation by which the expression of flagella is found in some cells but not in others of the same population [16]. The forth mechanism is the urease-mediated stone formation. Production of ammonia by the action of urease result in stone formation, and these stones in turn help to protect the bacteria [16].

Proteus mirabilis is a primary pathogen in urinary tract, and cause urinary tract infections. Infection by *P. mirabilis* occurs more commonly in patients with irregularly functioning urinary tract or in patients requiring long-term urethra catheterization [6].

As an opportunistic pathogen, urinary tract infections caused by this bacterium are more common in the elderly individuals [18].

The pathway for infection begins when *P. mirabilis* enters the urethra. The bacterium's motile abilities then allow movement and colonisation of the bladder and the kidneys. After colonising the bladder, *P. mirabilis* then produces fimbriae and adhesions that bind specifically to urinary tract mucosal surfaces. *Proteus mirabilis* also produce urease, which hydrolyses urea to ammonia, causing complication such as cystitis, acute pyelonephritis and formation of kidney and bladder stones [6] [8].

Current biotechnological research has focused on developing methods to prevent urinary tract infections caused by *P. mirabilis.* Several studies have tasted vaccines that use the bacteria's outer membrane, which the humoral immune system responds again. Future research should address ways to extend such methods of vaccination against other causes of urinary tract infection [19].

The bacteria can be found throughout the kidney stones, and this bacteria lurking in the kidney stone can reinitiate infection after antibiotic treatment. Once the stones develop, over time they may grow large enough to cause obstruction and renal failure. *Proteus* species can also cause wound infections, septicaemia, and pneumonia, mostly on the hospitalized patient. *Proteus mirabilis* in an alkaline urine sample can easily be diagnosed in the laboratory due to its characteristic swarming motility and inability to metabolise lactose on MacConkey agar plates. Also *P. mirabilis* produces a very distinct fishy odour [6] [20].

Proteus mirabilis makes several different fimbriae that promote adhesion to the mucosal surfaces. One of these fimbriae, called the mannose resistance *Proteus*-like fimbriae, has been highly present in patients associated with urinary tract infections [11].

A mannose resistance *Proteus*-like gene (MrpH) present in the *mrp* operon of *Mrp* fimbriae has been recently shown to be essential for functional adhesion of MR/p fimbriae [21].

Proteus mirabilis can be commonly present in healthy individual as part of the normal mucosa. The bacterium becomes a significant problem mostly in individual that have vulnerable immune system and are in danger of nosocomial transmission, such as hospital patients [22].

Current study shows that there are a number of antibiotics that were once effective against *P. mirabilis* that are now useless due to the extended spectrum beta lactamases (ESBLs). These are enzymes that pass through plasmids and are found in the most of the family *Enterobacteriaceae.* These plasmids are found within abscesses, blood, catheter tips, lungs, peritoneal fluid, sputum and throat culture. Detected in the 1980's in *Klebsiella* and *Esherichia coli*, these enzymes were found to hydrolyse antibiotic cephalosporin thus making it ineffective. The ESBL's become highly dangerous when produced in copious amounts, conveying resistance to a large number of antibiotics used. The spread of these plasmids is primarily prevalent in the healthcare facilities where patients have extended hospital stays, are using catheters, are within the intensive care unit (ICU), have had recent surgery or are administered consistently with antibiotic [22] [23].

MATERIALS AND METHODS

Collection of Specimen

Total of 100 early-morning, mid-stream urine specimens and urinary catheter swabs were randomly collected from patients attending Sickbay Hospital, Ahmadu Bello University, Zaria and transported immediately to Microbiology laboratory, Nigerian Institute of Leather and Science Technology, Zaria for processing.

Sample processing

The collected urine samples were centrifuged at the speed of 1500 rpm for five minutes. The supernatants were discarded, and the residues left (and the collected urinary catheters) were collected using non-pyrogenic swab sticks that were previously moistened with physiological saline, and aseptically inoculated on the surface of Cystein Lactose Electrolyte Deficient (CLED) agar, and incubated at the temperature of 37°C for 24 hours under aerobic condition. The culture plates were then examined after overnight incubation for the presence of swarming growth characteristic of the organism. A portion of the cultured colonies with swarming growth was picked and sub-cultured on the blood agar and incubated at the temperature of 37°C for 24 hours so as to obtain a pure culture. The suspected colonies were Gram stained and observed for the preliminary identification of *Proteus mirabilis*. Suspected *Proteus mirabilis* colonies were isolated and characterized biochemically using Citrate, Urease, Motility and Methyl Red, Indole and Voges-Proskauer tests, as well as triple sugar iron utilization test as adopted by Baker [24].

Biochemical identification of the isolated *Proteus mirabilis*

All isolates suspected to be *Proteus mirabilis* on the basis of their cultural appearance, preliminary microscopic identification and morphological characteristics were identified biochemically using various tests.

a. Triple Sugar Iron (TSI) Test

The triple sugar iron was prepared in such a way that there is slant and the butt. With a sterilized straight inoculating needle, the isolated colony was picked from the solid media and stabbed the TSI medium up to the butt and then streaked the surface of the agar slant. The cap was left loosely and incubated at temperature of 37°C for 24hours.

After the incubation, black precipitates were observed on the TSI and also a reddish or yellowish colouration to signify acidic or basic utilization. The appearance of blacked butt indicates hydrogen sulphide production (H_2S) [25].

b. Citrate Test
The agar was prepared in a slant form. A colony of the isolated bacteria was picked and inoculated on Simmon's citrate agar slightly on the slant surface by touching the tip of the media and then incubated at 37°C for 24hours in such a way that after incubation, a blue precipitate observed as the positive result [25].

c. Urease Test
The urease agar slants were inoculated with a portion of the bacterial colony, and the slant was incubated at 37°C for 24 hours. If the organism produced urease enzymes, the colour of the slant changes from light orange to magenta. But if the organisms did not produce urease, the slant and the butt remains orange in colouration [25].

d. Indole Utilization Test
This is used to determine the ability of an organism to split amino acid tryptophan to form compound called indole. The tryptophan was inoculated with emulsified isolated colony of the test organism in the tryptone broth. The set up was incubated at the temperature of 37°C for 24hrs, after which a 0.5ml of Kovac's reagent was added to the broth culture. The Indole reagent retained its yellow colour indicating a negative test. While a positive reaction was determined by the development of a red coloured ring on the reagent layer floating on the broth within one minute [25] [26].

e. Methyl Red and Voges Proskauer (MR-VP) Tests
About 5ml of glucose broth was inoculated and incubated for 48 hours at 37°C, after the period of incubation, 1ml of the broth was transferred to a small serological test tube, and 2 drops of methyl red was added. Following addition of the indicator, a red colour precipitate signifies positive methyl red test. While a yellow colour precipitate signifies a negative test. A heavy inoculum of the test organism was inoculated into Voges-Proskauer medium contained in different test tubes. The tubes were incubated at 37°C for 48 hours. After which 0.5ml of alpha-naphthol was added followed by 0.5ml of 40% KOH. It was then agitated and allowed to stand for 30 minutes; a red to pink colour signifies a positive test [25] [26].

f. Motility Test
The motility medium in a test tube was inoculated by stabbing to a depth of 2mm. The tubes were incubated at 37°C for 24 hours. A positive result was indicated by a cloudy and distinct line of inoculation. A negative result was identified with sharp indistinct inoculation line [25] [26].

Antimicrobial Susceptibility Test
Susceptibility to antibiotic agents was determined by using disk-diffusion method. From the Nutrient agar plate, bacterial colonies were transferred into McCartney bottles containing sterile normal saline to obtain bacterial density of 3×10^8 organisms per milliter as determined by McFarland standard scale number 1. The culture was streaked uniformly onto freshly prepared Nutrient agar plates using disposable sterile swabs. The plates were allowed to dry briefly, and then discs of multiple antimicrobials were mounted on the surface of the streaked inoculums. The plates were incubated at 37°C for 24 hours. Following overnight incubation, the culture plates were examined for the evidence of inhibition. A meter rule was used to measure the zones of growth inhibition [25] [26] [27]. The isolates were recorded as slightly sensitive, moderately sensitive, highly sensitive or resistant to the corresponding antibiotics by comparing the values obtained with the recommended standard charts given by NCCLS [28] [29]. Antibiotic susceptibility patterns of the isolated organisms were therefore tested against some commonly used antibiotics, which include; Streptomycin (S), Sparfloxacin (SP), Ciprofloxacin (CPX), Gentamicin (CN), Septrin (SXT), Tarvid (OFX), Pefloxacin (PEF), Zinnacef (Z), Rocephin (R), Ampiclox (APX), Augumentin (AU), Amoxicillin (AM), Erythromycin (E) and Tetracycline (TET).

RESULTS AND DISCUSSION

TABLE 1: shows the prevalence of *Proteus mirabilis* in the study area. The total prevalence of the bacteria was found to be 20% out of the total 100 samples analyzed.

TABLE 2: shows the biochemical characterization of the isolated *Proteus mirabilis*. The predominant of the suspected isolates following preliminary cultural and microscopic analysis were found to be *Proteus mirabilis* species.

TABLE 3: Shows the antibiotic susceptibility pattern of the isolated *Proteus mirabilis*. Streptomycin, Sparfloxacin, Ciprofloxacin, Pefloxacin and Erythromycin recorded high activity against the bacterium. While, the isolated bacteria were found to be relatively resistant to antibiotics such as: Gentamicin, Augumentin, Amoxicillin, Zinnacef, Rocephin and Ampiclox.

Table 1: Prevalence of *Proteus mirabilis* in the study area.

Isolates	POSITIVE	NEGATIVE	Prevalence (%)
Presence of *Proteus mirabilis*	20	80	20%
Absence of *Proteus mirabilis*	80	20	80%
Total	100	100	**100**

Table 2: Biochemical characterization of the isolated *Proteus mirabilis.*

ISOLATE	TSI	CITRATE	UREASE	MOTILITY	MR	VP	INDOLE	INFERENCE
SP.13	$R/_{Y}$+H_2S	+	+	+	+	-	-	*P. mirabilis* was isolated
SP.20	$R/_{Y}$+H_2S	+	+	+	+	-	-	*P. mirabilis* was isolated
SP.25	$R/_{Y}$+H_2S	+	+	+	+	-	-	*P. mirabilis* was isolated
SP.27	$R/_{Y}$+H_2S	+	+	+	+	-	-	*P. mirabilis* was isolated
SP.37	$R/_{Y}$+H_2S	+	+	+	+	-	-	*P. mirabilis* was isolated
SP.41	$R/_{Y}$+H_2S	+	+	+	+	-	-	*P. mirabilis* was isolated

Key: MR= methyl Red; VP= Voges Proskauer; R=Red; Y=Yellow; H_2S=hydrogen sulfide; TSI= Triple Iron Sugar

Table 3: Antibiotic susceptibility trends of the isolated *Proteus mirabilis*.

ISOLATES	ANTIBIOTICS	DISC POTENCY	RESISTANCE	SLIGHTLY SENSITIVE	MODERATE SENSITIVE	HIGHLY SENSITIVE
1	Streptomycin	30 µg	0(0%)	0(0%)	0(0%)	20(100%)
2	Sparfloxacin	10 µg	2(10%)	1(5%)	2(10%)	15(75%)
3	Ciprofloxacin	10 µg	0(0%)	3(15%)	3(15%)	14(70%)
4	Chloramphenicol	30 µg	6(30%)	10(50%)	3(15%)	1(5%)
5	Septrin	30 µg	10(50%)	4(20%)	3(15%)	3(15%)
6	Tarvid	10 µg	2(10%)	10(50%)	4(20%)	4(20%)
7	Pefloxacin	30 µg	2(10%)	1(5%)	3(15%)	14(70%)
8	Gentamicin	10 µg	8(40%)	8(40%)	4(20%)	0(0%)
9	Augumentin	30 µg	17(85%)	2(10%)	1(5%)	0(0%)
10	Amoxicillin	30 µg	20(100%)	0(0%)	0(0%)	0(0%)
11	Zinnacef	20 µg	16(80%)	4(20%)	0(0%)	0(0%)
12	Rocephin	25 µg	8(40%)	11(55%)	1(5%)	0(0%)
13	Ampiclox	30 µg	18(90%)	2(10%)	0(0%)	0(0%)
14	Erythromycin	10 µg	0(0%)	0(0%)	3(15%)	17(85%)
15	Tetracycline	15 µg	19(95%)	1(5%)	0(0%)	0(0%)

Based on the findings of the research study, out of the total of 100 samples analysed, 20 samples recorded the occurrence of *Proteus mirabilis* which accounted for the prevalence of 20% (table 1). This agrees with the findings of Coker *et al.*, [6] who reported that some believed that *Proteus mirabilis* has access to bladder by infecting the peri-urethral area. But, the findings deviated from that of Gonzales [3], who reported that the prevalence of *Proteus mirabilis* infections is 90%. This may be due to the immune status, environmental factors or genotypic factors of the various populations studied.

Based on the biochemical characterizations, the isolates that showed positive reaction to Citrate, Urease, Motility, Methyl Red and Triple Sugar Iron, but negative reaction to Indole and Voges-Proskauer were identified as *Proteus mirabilis*. This confirmation was done following preliminary identification using cultural appearance and microscopy (Table 2).

Based on the antibiotic susceptibility pattern of the isolated *Proteus mirabilis*, the bacterium was found to be highly sensitive to Streptomycin 20 (100%); Erythromycin 17 (85%); Ciprofloxacin 14 (70%) and Sparfloxacin 15 (75%). But, it was found to be resistant to Amoxicillin 20 (100%); Tetracycline 19 (95%) and Zinnacef 16 (80%) (Table 3). This conforms with the work of Luzzaro *et al*., [20], Isenberg [30] and Lyon *et al.*, [30], who reported that *Proteus mirabilis* species are resistant to Tetracycline and beta-lactam antibiotics such as Penicillin and Amoxicillin.

CONCLUSION

The prevalence of *Proteus mirabilis* in the study area was found to be 20%. About 20% of urinary tract infections in the study area are caused by *Proteus mirabilis*.

Based on the findings of the research, the bacterium (*Proteus mirabilis*) was found to be sensitive to Streptomycin, Erythromycin, Sparfloxacin and Pefloxacin, all of which can be used as first line drugs of choice in the treatment of infections caused by *Proteus mirabilis*. The organism is resistant to Tetracycline, Amoxillin and Ampiclox.

From the work done, multiple drug resistance strains of *Proteus mirabilis* are the common causative agents of urinary tract infections. It is recommended that continuous surveillance of antimicrobial susceptibility of clinical isolates of *Proteus* species is of importance to keep in check the antimicrobial pattern of such isolates.

Proper hygiene needs to be adopted seriously in health institutions like clinics and hospitals, since *Proteus mirabilis* is highly nosocomial that can spread from reservoirs to healthy persons.

Also, regular researches have to be maintained to determine the emergence of antibiotic resistance by *Proteus mirabilis* as soon as it is in existence.

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