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Identification and Characterization of Proteus Species from Urine Samples in Federal Medical Center (FMC) Yenagoa, Bayelsa State, Nigeria

Tolulope O Alade*. Omonibo E. F. Raphael E. Mbam

Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Nigeria

**Corresponding author*

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A B S T R A C T

Urinary tract infections has remained a public health challenge and *Proteus* species have been implicated as the second commonest cause after *Escherichia coli* and *Klebsiella* species. *Proteus* species belong to the family of Enterobacteriaceae. Pathogenicity of *Proteus mirabilis* is enhanced by their possession of unique virulence factors which it uses to colonize the host urinary tract, including urease and stone formation, adhesions/fimbria, toxins, and hemolysin. The aimed at identifying and characterizing *Protues species* from urine samples of patients attending Federal Medical Center Yenagoa (FMCY). A total of 50 urine samples were collected and analyzed using standard phenotypic and 16S rRNA amplification techniques. Of the 50 urine samples collected, 18(36%) were collected from males while 32(64%) from females. Four (4) bacterial isolates were analyzed by 16S rRNA gene sequencing, and the phylogenetic tree was constructed to ascertain *Proteus species* to its species level. The antimicrobial susceptibility pattern showed that out of the 7(14%) isolates of *Proteus species* 3(42.86%), 7(100%), 6(85.71%), 1(14.29%), 5(71.43%), 7(100%), 0, 0, 6(85.71%) and 2(28.57%) were resistant to (Sulfamethaxozole-trimethoprim), (Chloramphenicol), (Sparfloxacin), (Ciprofloxacin), Ampicillin, (Augmentin), (Gentamicin), Perfloxacin, (Tarivid), and (Streptomycin). Results from the study showed that the molecular characterization confirmed the identity of the organism as was obtained from the phenotypic characterization of the isolates. The study revealed widespread resistance to most routine antimicrobials, which calls for proper surveillance against Proteus species.

Introduction

Proteus species are grouped among the Enterobacteriaceae family of gram-negative bacilli. They have the ability to ferment maltose but are not able to utilize lactose as source of sugar. They also belong to the group of bacteria referred to as facultative anaerobes. The credit for the first isolates goes to reports and characterization by Hauser in the late 19th century (Slattery *et al.,* 2020). The genus is currently composed of *Proteus mirabilis, Proteus vulgaris, Proteus penneri, Proteus hauseri, Proteus terrae,* and *Proteus cibarius* (Adebo *et al.,* 2023). The species most implicated in clinical isolates include *P mirabilis* and *P vulgaris*. *Proteus* organisms are implicated as serious causes of infections in humans, along with *Escherichia, Klebsiella,*

Enterobacter, and *Serratia* species (Sun *et al.,* 2019).

Proteus species are found as part of the normal microbiota of the intestinal tracts of humans, along with *Escherichia coli* and *Klebsiella* species, of which *E coli* is the predominant resident. Other areas *Proteus* is found include multiple environmental habitats, such as long-term care facilities and hospitals (Potron *et al.,* 2019). Though in hospital settings, it is not unusual for gram-negative bacilli to colonize both the skin and oral mucosa of both patients and hospital personnel (Mahabubul *et al.,* 2018). Infection primarily occurs from these reservoirs. Yet, *Proteus* species are not the most common causative pathogens of nosocomial infections. Nearly 90% of *Proteus* infections are caused by *P mirabilis* and can be considered a community-acquired infection. *P. vulgaris* and *P. penneri* may be isolated from individuals in long-term care facilities and hospitals and from patients with underlying diseases or compromised immune systems (Mahabubul *et al.,* 2018).

Proteus species have some virulence determinants such as an extracytoplasmic outer membrane, a feature shared with other gram-negative bacteria (Mahabubul *et al.,* 2018). In addition, the outer membrane contains a lipid bilayer, lipoproteins, polysaccharides, and lipopolysaccharides (Pelling *et al.,* 2019). The ability of Proteus species to cause an infection depends on the interaction between the infecting organism and the host defense mechanisms. Various components of the membrane interplay with the host to determine virulence. Inoculum size is important and has a positive correlation with the risk for infection (Mahabubul *et al.,* 2018).

The ability of the microbe to adhere to the host tissue initiates the beginning of an infection. Fimbriae facilitate adherence and thus enhance the capacity of the organism to produce disease. *E coli*, *P mirabilis*, and other gram-negative bacteria contain fimbriae (i.e, pilli), which are tiny projections on the surface of the bacterium. Specific chemicals located on the tips of pilli enable organisms to attach to selected host tissue sites (eg, urinary tract endothelium) (Enam, 2011). The presence of these fimbriae has been demonstrated to be important for the attachment of *P mirabilis* to host tissue (Mahabubul *et al.,* 2018).

The attachment of *Proteus* species to uroepithelial cells initiates several events in the mucosal endothelial cells, including secretion of interleukin 6 and interleukin 8. *Proteus* organisms also induce apoptosis (programmed cell death) and epithelial cell desquamation (Norsworthy, et al., 2017; Odoki *et al.,* 2019). Bacterial production of urease has also been shown to increase the risk for pyelonephritis in experimental animals. Urease production, together with the presence of bacterial motility and fimbriae, may favor the production of upper urinary tract infections (UTIs) by organisms such as *Proteus* (Pavez *et al.,* 2019). The genitourinary tract is the site of disease responsible for gram-negative bacteremia in approximately 35% of patients. Bacteriuria occurs in 10-15% of hospitalized patients with indwelling catheters. The risk for infection is 3-5% per day of catheterization (Slattery *et al.,* 2020).

Urinary tract infections are the second commonest infection that necessitates hospital admission, second to pneumonia. Untreated or mistreated urinary infections can lead to sepsis (Pelling *et al.,* 2019). And because there are several key differences between *P. mirabilis* UTI and uropathogenic *Escherichia coli* UTI, including urolithiasis, bacterial metabolism during UTI, and intracellular versus luminal niches in the bladder (Chelsie *et al.,* 2018). *P. mirabilis* induces a pro-inflammatory response during UTI; Humans infected with *P. mirabilis* have elevated levels of CXCL1 at 72 h postinfection (hpi) and interleukin-10 (IL-10) at 6 and 96 hpi in their urine (Majeed *et al.,* 2019).

Notably, the cytokine response is however further increased during co-infection with *P. stuartii*, with increased levels of CCL2, CCL5, CXCL1, IL-6, IL-10, IL-17A (Chelsie *et al.,* 2018), tumor necrosis factor alpha (TNF-α), beta interferon (IFNβ), and IFNγ at 48 hpi.

Against this order, *P. stuartii* infection did not lead to increased levels of any of the measured cytokines (Chelsie *et al.,* 2018). Some of the elevated cytokines, such as IL-10, are anti-inflammatory (Chelsie *et al.,* 2018), suggesting there are switches between pro- and anti-inflammatory responses as the infection progresses (Okeefe *et al.,* 2019). Several anitbiotics such as Sulfamethaxozole-trimethoprim, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Ampicillin, Augumentin, Gentamicin, Pefloxacin, Tarivid, and Streptomycin were very widely utilized in the treatment of urinary tract infection (Chelsie *et al.,* 2018). However, various reports have revealed resistance to most of these antimicrobials. Therefore, the study focused on the proper isolation and identification of *Proteus* species while establishing their baseline antimicrobial susceptibility profile.

Materials and Methods

A total of 50 urine samples were collected from Federal Medical Centre, Yenagoa. The bacterial isolates were identified using a standard bacteriological technique, the isolates were analyzed by 16SrRNA gene sequencing, and the phylogenetic tree was constructed to ascertain *Proteus species* to its species level while the antibiotic susceptibility testing was done by disc diffusion.

Results and Discussion

The study showed that, of the 50 urine samples collected, 18(36%) were collected from males while 32(64%) from females. Seven (7) bacterial isolates were realized and analyzed by 16S rRNA gene sequencing and which were *Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae*, and the phylogenetic tree was constructed to ascertain *Proteus species* to its species level. The antimicrobial susceptibility pattern showed that out of the 7(14%) isolates of *Proteus species* 3(42.86%), 7(100%), 6(85.71%), 1(14.29%), 5(71.43%), 7(100%), 0, 0, 6(85.71%) and 2(28.57%) were resistant to (Sulfamethaxozole-trimethoprim), (Chloramphenicol), (Sparfloxacin), (Ciprofloxacin), Ampicillin, (Augmentin), (Gentamicin), Perfloxacin, (Tarivid), and (Streptomycin).

The rising incidences of urinary tract infections in patients attending various healthcare facilities in Nigeria calls for proper and timely surveillance against any agent of hospital and/or community acquired infections. This is made worse by the rising pace of widespread resistance against most life-saving antimicrobials. This study not only aimed at identifying the phenotypic and molecular characterization of *Proteus* species, but even more so established the antimicrobial susceptibility profile of the isolated species. The study revealed that the molecular characterization of the *Proteus* species and the phenotypic identification of the bacterial isolates had more of *Proteus mirabilis,* which is mostly implicated in most urinary tract infections. This agrees with the reports of Slattery *et al.,* (2020), which revealed that *Proteus* is the second most implicated organism in urinary tract infection after *Escherichia coli* and *Klebsiella pneumonia.*

The study also revealed higher incidence of *Proteus* species in older individuals and less in the younger. This could be explained in the context of higher innate immunity in the younger adults than in the older people. And secondly, acquired resistance due to re-occuring infections may have been a factor in higher incidences. The antimicrobial susceptibility profile revealed that most of the *Proteus* species are resistant to such drugs that were initially lethal to them such as Sulfamethaxozole-trimethoprim, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Ampicillin, Augmentin, Gentamicin, Perfloxacin, Tarivid, and Streptomycin. This report agrees so well with the study by Kranz *et al.,* (2018), which revealed possession of multidrug efflux pump by most gram negative bacteria, a critical factor in conferring resistance to these pathogens against most available antimicrobials.

The study therefore shows that various *Proteus* species are implicated in urinary tract infections, especially *Proteus mirabilis* and given the widespread resistance to antimicrobials, there is the need for proper surveillance against *Proteus* species and timely treatment to minimize the increasing pace of antimicrobial resistance.

The study revealed a significant incidence of *Proteus* species in clinical urine isolates and showed a significant resistance to most antimicrobials and given the roles of this pathogen in the causation of urinary tract infection, there is an increasing need for periodic surveillance against the pathogen and timely and appropriate treatment, following isolation and identification.

Table.1 Distribution of Specimen by Age and Gender (Male/Female)

Age range (yrs)	Male%	Female%	Total%
10-19	8(40%)	12(60%)	20(40%)
20-29	6(40%)	9(60%)	15(30%)
30-39	3(50%)	3(50%)	6(12%)
40-50	1(11.11%)	8(88.89%)	9(18%)
Total	18(36%)	32(64%)	50(100%)

Table.2 Distribution of Bacterial Isolates by Age amongst Male Subjects

Age range (yrs)	*K. pneumoniae*	*P. mirabillis*	*P. aeruginosa*	*E.coli*	Total(%)
10-19	5(62.5%)	-	-	3(37.5%)	**8(44.44%)**
20-29	-	-	2(66.67%)	1(33.33%)	**3(16.67%)**
30-39	1 (33.33%)	1(33.33%)	1(33.33%)	-	**3(16.67%)**
40-50	-	1(25%)	-	3(75%)	**4(22.22%)**
Total	6 (33.33%)	2(11.11%)	3(16.67%)	7(38.89%)	**18(100%)**

Table.3 Distribution of Bacterial Isolates by Age amongst Female Subjects

Age range (yrs)	*K. pneumoniae*	*P. mirabillis*	*P. aeruginosa*	*E.coli*	Total(%)
10-19	4(33.33%)	**---------**	2(16.67%)	6(50%)	**12(37.5%)**
20-29	2(22.22%)	**--------**	----------	7 (77.78%)	**9(28.13%)**
30-39	2(50%)	**1(25%)**	----------	1(25%)	**4(9.37%)**
40-50	-------	**4(57.14%)**	2(28.57%)	1(14.29%)	**7(25%)**
Total	8(18.75%)	**5(21.88%)**	4(12.5%)	15(46.87%)	**32(100%)**

Table.4 Gram Stain Reaction and Biochemical Tests

Test	PM	PM	PM	PM	PV	PM	PV
Gram reaction	GNB	GNB	GNB	GNB	GNB	GNB	GNB
Citrate	+	+	+	+	+	+	+
Indole	-	-	-	-	+	-	+
KIA	Alkaline slant/acid butt	Alkaline slant/acid butt	Alkaline slant/acid butt	Alkaline slant/acid butt	Alkaline slant/acid butt	Alkaline slant/acid butt	Alkaline slant/acid butt
Methyl red	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+
Motility test	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-
H_2S	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+
VogesProskauer	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+
Gelatin hydrolysis	-	-	-	-	-	-	-
ONGP	-	-	-	-	-	-	-

KEYS:
KIA; Kligler's Iron Agar, **+ve**:Positive, **-ve**:Negative **ONPG** = o-nitrophenyl-b-D-galactopyranoside (β-galactosidase), **H_2S**: hydrogen sulfide production, **PM:** *Proteus mirabilis,* **PV:** *Proteus vulgaris,* **GNB:** Gram negative bacilli

Figure.1 Distribution of Bacterial Isolates

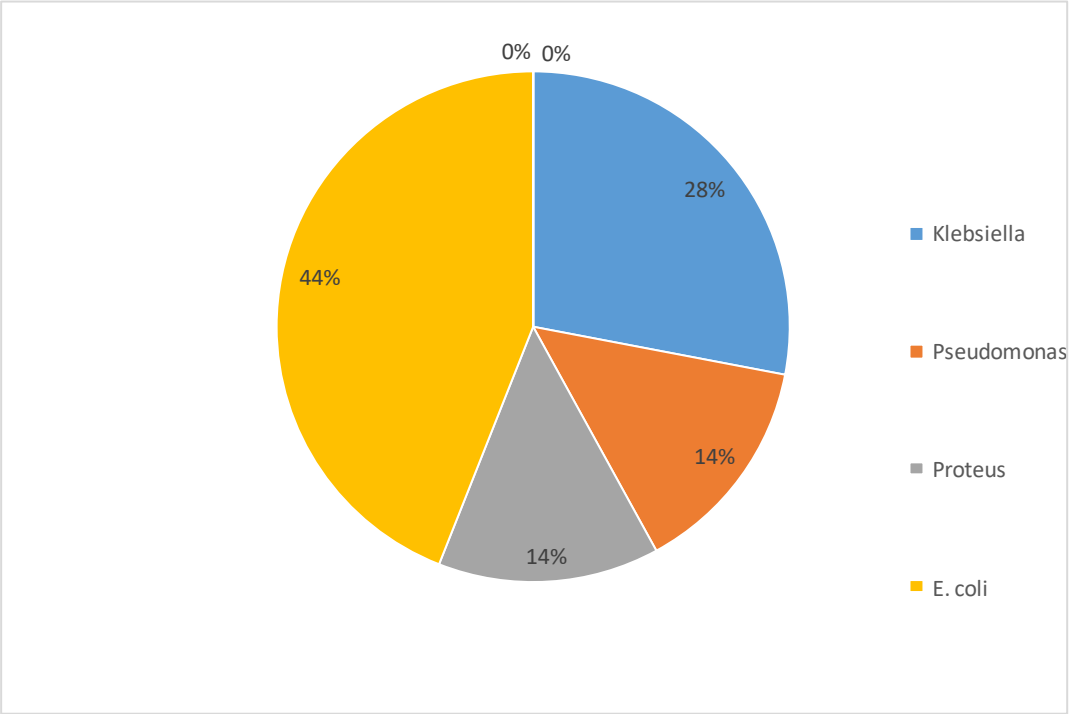


Figure.2 Phylogenetic Tree Showing the Evolutionary Distance between the Bacterial Isolates.

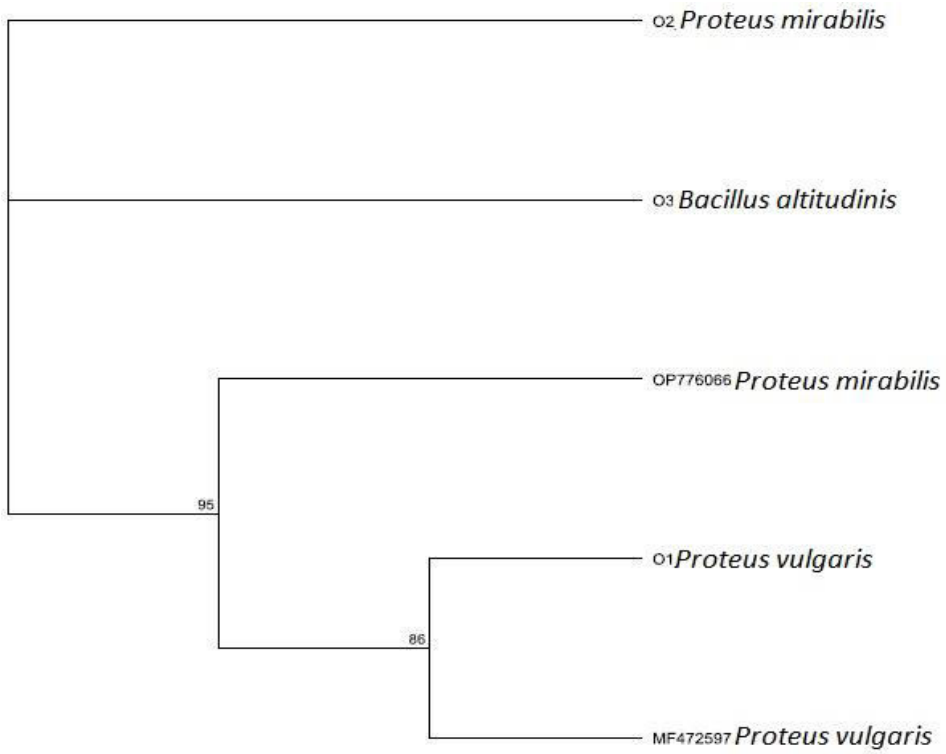
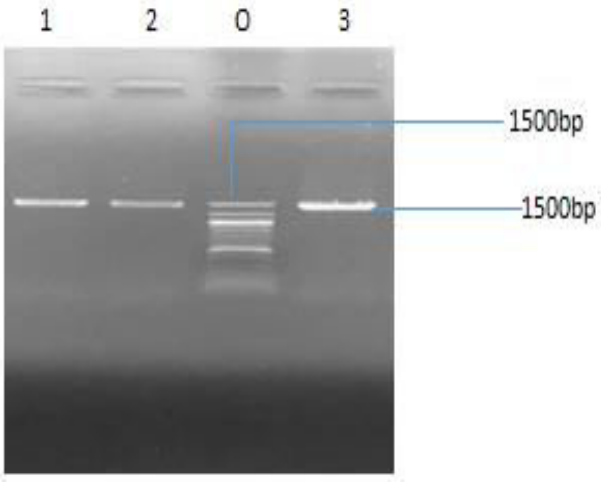


Plate.1 Agarose gel electrophoresis of 16SrRNA gene of some selected bacterial isolates



Agarose gel electrophoresis of selected bacterial isolates.
Lanes 1 – 3 represent 16SrRNA gene bands (1500bp).
Lane O represents the 100bp DNA ladder.

Author Contribution

Tolulope O Alade: Investigation, formal analysis, writing—original draft. Omonibo E. F. Raphael E. Mbam: Validation, methodology, writing—reviewing-Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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