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# *UreC* and *ZapA* virulence genes amplification in clinical specimen of *Proteus mirabilis* in Bayelsa state, Nigeria

## Abstract

**Background and objective:** Proteus mirabilis is part of the Enterobacteriaceae family, Gram negative bacterium which typically lives in the human gut, which means when it causes illness it can be a serious bloodstream infection, urinary tract infection or disseminated infection. UreC and ZapA virulent genes constitute the major pathogenicity of this organism as well as its resistance to antibiotics. This study was carried out to detect the presence of UreC and ZapA genes in *Proteus mirabilis* isolates sourced from Federal Medical Centre and Niger Delta University teaching hospital in Bayelsa State.

**Materials and methods:** A total of one hundred and forty (140) clinical samples were collected from Federal Medical Centre (FMC) Yenagoa and Niger Delta University Teaching Hospital (NDUTH) Okolobiri, Bayelsa State. Of the 140 samples collected, 64(45.7%) were from males while 76(54.3%) were from females. The samples were Urine, Sputum, High vaginal swab, Urethral swab, Ear swab and Wound swab. The samples were inoculated in different laboratory media and incubated at 37°C for 48 hours. Morphological, cultural, biochemical characteristics and Polymerase Chain Reaction (PCR) technique were noted appropriately. Means and corresponding standard deviations were calculated for continuous data while proportions, along with the 95% confidence intervals, were calculated for categorical data.

**Results:** A total of 81 bacterial isolates were obtained from these samples, of which 17(20.9%), 22(27.2%), 8(10.0%), 10(12.3%) and 24(29.6%) were *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* respectively. Fifteen (15) out of the 17(20.9%) *P. mirabilis* isolates were subjected to single-plex PCR amplification using specific primers after extraction of bacterial DNA from the samples. Out of the 15 samples, 14(93.3%) were positive for UreC gene while 15(100%) were positive to ZapA gene.

**Conclusion**: The present study revealed that virulent genes-UreC and ZapA are highly present in *P. mirabilis* isolates obtained from clinical specimens from FMC and NDUTH in Bayelsa state, thus making them more pathogenic and resistant to antibiotics curing effect.

**Keywords:** *UreC*, *ZapA*, *Proteus mirabilis*, NDU, Bayelsa State

Volume 9 Issue 1 - 2021

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**Received:** May 20, 2020 | **Published:** February 25, 2021

## Introduction

The persistence of an infection even after supposedly proper treatment could be as a result of wrong diagnosis, medication errors, rational choice of drugs, postulate treatment outcomes, drug interactions, and potential adverse drug reactions, low level of your immunity and resistance of the antimicrobials by the etiologic agents.

If the persistence of the infections is central to the resistance of the antimicrobial agents which is the failure of microorganisms notably bacteria, fungi, parasites viruses to resist the effects of medications that were previously used to treat them and the resistance faced by medications notably antibiotics that had previously treated an infection of bacteria origin termed antibiotic resistance (ABR), then there is a need to look closely at these central resistance genes.

Pathogenic bacteria have worked out many different ways to overcome the host defense system. A number of biological features known as virulence factors are common to many bacterial species, although some of these are characteristic only for certain bacteria.[1] The genus Proteus is a Gram-negative bacillus that belongs to the Enterobacteriaceae family. Members of the genus Proteus are widespread in the environment and the gastrointestinal tract of human and animals.[2] Proteus is known as nosocomial, opportunistic pathogen and is more common in community-acquired infections.[3]

Proteus mirabilis is one of the most common causes of UTI in individuals with long-term indwelling catheters, or complicated UTIs, and of bacteremia among the elderly (Liaw *et al*., 2004).[4,5] It is a Gram-negative rod shaped bacterium, well-known for its urease production and distinctive ability to differentiate into elongated swarm cells and characteristic bull's-eye pattern of motility on agar plates. It belongs to the class- Gammaproteobacteria, order-Enterobacteriales, family-Enterobacteriaceae, genus-Proteus, and specie- mirabilis.[6] It is a non-lactose fermenter organism, positive to urease test, citrate utilization test, hydrogen sulphide production test and phenylalanine deaminase test, but negative to indole, and sucrose fermentation tests.[7] It is an opportunistic bacterial pathogen which under favorable conditions causes many diseases such as UTIs, and especially with complicated UTI.[8] Usually, they affect the upper part of urinary tract causing infections such as cystitis, urolithiasis (kidney or bladder stones), and acute pyelonephritis and occasional cases of neonates or infants' meningitis, bacteremia, wound infections, septicemia, and rheumatoid arthritis.[9] *P. mirabilis* expresses several virulence factors

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involved in uropathogenesis such as adhesions, swarming motility, urease, hemolysin, proteases, and lipopolysaccharide endotoxins.[10] *UreC* encodes the large subunit {the urea-inducible urease gene cluster (ureRDABCEFG)} responsible for the production of urease enzyme by P. mirabilis, and it is very highly conserved among all species, so is regarded as a diagnostic feature of *P. mirabilis*.[11,12] Urease assists *P. mirabilis* to develop bacteriuria, cystitis, as well as kidney, and bladder stones.[13]

ZapA (mirabilysin/serralysin) is a metalloprotease capable of mediating the degradation of numerous host proteins in vitro. ZapA has been considered to have a role as virulence factor for P. mirabilis. However, its role in the pathogenicity of this bacterium is associated with the hydrolysis of IgA, which would destroy an important component of the host defense system of mammalian mucous surfaces. It is possible, however, that the enzyme may be effective for microbial proliferation, destroying other bioactive molecules like defensins, involved in the innate defense, or structural components of the host cells like matrix proteins.[14]

## Materials and methods

### Study design

This was a Cross-sectional study involving the use of quantitative methods for data collection in Yenagoa and Okolobiri, Bayelsa State.

### Study area

This study was carried out in Federal Medical Center (FMC), Yenagoa and Niger Delta University Teaching Hospital (NDUTH), Okolobiri, Bayelsa State. Bayelsa State is a multicultural state in the southern part of Nigeria in the core Niger Delta region. Geographically, Bayelsa State is located within Latitude: 04° 15' North, 05° 23' South and Longitude: 05º 22' West and 06º 45' East. It shares boundaries with Delta State on the North, Rivers State on the East and Atlantic Ocean on the West and South and is populated by different ethnic groups across the country.

### Ethical Clearance

Ethical clearance was obtained from the ethical committee of Niger Delta University Teaching Hospital (NDUTH), Okolobiri and Federal Medical Center (FMC), Yenagoa.

### Specimen collection

A total of one hundred and forty (140) samples were obtained from the Microbiology Department of Niger Delta University Teaching Hospital (NDUTH), Okolobiri and Federal Medical Center (FMC), Yenagoa, Bayelsa State. Sterile swabs sticks and containers (commercially obtained) were used for sample collection. Large mouth sterile containers were used for urine and sputum samples collection; sterile swab sticks moistened with normal saline were used for high vaginal swabs (HVS), urethral swabs, ear swabs and wound swabs samples collection. Culture media used were MacConkey agar (Oxoid), Chocolate agar (Oxoid) and Nutrient ager (oxoid). Swab sticks were dipped into peptone water before 24 hrs of incubation at 37°C. One (1) ml of the broth culture was inoculated onto prepared plate of mac Conkey agar, Nutrient agar and chocolate agar. The plates were incubated for 18 hours and the pure cultures of the isolates stored on nutrient agar slant at 4°C until needed.

### Antibiogram

This was carried out by agar disc diffusion method using commercially available discs to determine susceptibility pattern of the isolates. The antibiotics used were Tarivid (OFX), Reflacine (PEF), Ciprofloxacin (CPX), Augmentin (AU), Ampicillin (PN), Gentamycin (CN), Streptomycin (S), Ceporex (CEP), Nalidixic acid (NA), Cotrimoxazole (SXT), Amoxicillin (AML), Norfloxacin (NB), Rifampicin (RD), Erythromycin (E), Ampiclox (APX), Levofloxacin (LEV) and Chloramphenicol (CH). Nutrient agar plate was flooded with peptone broth containing the isolates; excess broth on the agar was drained off and the antibiotic disc was then placed on the surface of the agar and incubated at 37ºC for 24hours. Sensitivity/susceptibility to a particular antibiotic was indicated by a clear zone of inhibition around it. The zones of inhibition were measured by the diameter in millimeters (mm) using a meter rule (Gary, 2011).

### Molecular analysis

#### DNA extraction (Boiling Method)

An overnight culture of the pure isolate in peptone broth was transferred into a 2ml Eppendorf tube and was spun at 14000rpm for 3minutes in a Denville 260D brushless micro-centrifuge manufactured by Denville Scientific Incorporated. The supernatant was then discarded and 1000µl of 0.5% normal saline was added to the sediment and was vortexed on el tech XH-B vortex. The tube was inserted into a heating block at 95ºC for 20minutes, after which it was fast cooled in a freezer for 10minutes. On cooling, the tube was spun again at 14000rpm for 3minutes and 500µl of the supernatant (containing the bacterial DNA) was aspirated and dispensed into a 1.5ml Eppendorf tube and was stored in the freezer at -2ºC for preservation and further analysis.

#### DNA quantification

Quantification of the extracted bacterial DNA was done using a Nanodrop 1000 spectrophotometer. Two (2)µl of the extracted bacterial DNA was placed on the lower pedestal using an automated micropipette and the higher pedestal was dropped at that same spot. The quantity and purity of the DNA was read off the computer.

#### Amplification of *UreC* and *ZapA* gene

The PCR mix comprised X2 dream taq master mix (Taq polymerase, dNTPs, MgCl, buffer), the primers (forward and reverse) at a concentration of 0.4µM (supplied by Inqaba Biotech, South Africa), template (the extracted bacterial DNA), and water. Each PCR amplification process was done in 35cycles and is summarized below (table 1 & 2).

**Table 1** Programs of PCR thermocycling conditions

| Amplification processes | UreC gene | ZapA gene |
|---|---|---|
| Initial denaturation | 95°C–5minutes | 95°C–3minutes |
| Final denaturation | 95°C–30seconds | 95°C–1minutes |
| Annealing | 54°C–30seconds | 60°C–1minutes |
| Initial extension | 72°C–30seconds | 72°C–1minutes |
| *35 cycles repeated* | | |
| Final extension | 72°C–3minutes 30secs | 72°C–5minutes |
| Infinity (∞) allow to cool | 10°C forever | 4°C forever |

#### Agarose gel electrophoresis:

A 5µl aliquot of each amplicon was resolved on 1.5% Agarose gel electrophoresis tinted with Ethidium Bromide at 120V for 30minutes and was visualized with an ultraviolet trans-illuminator. The sizes of the DNA were determined using a Quick load 1000bp Molecular DNA ladder.

## Results

Table 3 shows that majority of the respondents were females 76(54.3). However, comparing the High Vaginal Swab and Urethral Swab, the male respondents recorded more samples.

Table 4 shows that respondents within the ages of 21-30 recorded the highest rate of infection 18(45.0%), closely followed by the age bracket of 31-40 -8 (20.0%) and the lowest among the elderly 51-60-3(7.5%).

The highest number of isolates 16 (39.0%) was recorded within the age bracket of 21-30 with the lowest 1(2.4%) among the female respondents in table 5. *E. coli* constitutes majority of the isolates with a staggering 12(29.3%) and the lowest being *K. pneumoniae* 4(10%).

Urine sample constitutes the highest number of specimen 61(75.5%); the highest isolate recovered being *S. aureus* -24(29.6%); Urethra swab constitutes the lowest number of sample 2(2.5%) and the lowest isolate being *P. aeruginosa* 8(10.0%) (Table 6 & Table 7).

Plates 1&2: Agarose Gel Electrophoresis (Figure 1).

**Table 2** The sequence primers of *UreC* and *ZapA* genes (Ali and Yousif, 2015).

| Genes | Primer sequences (5'-3') | Size of base pair |
|---|---|---|
| *UreC* | Forward: GTT ATT CGT GAT GGT ATG GG | 317 |
| | Reverse: GTA AAG GTG GTT ACG CCA GA | |
| *ZapA* | Forward: ACC GCA GGA AAA CAT ATA GCC C | 533 |
| | Reverse: GCG ACT ATC TTC CGC ATA ATC A | |

**Table 3** Sex of respondents

| Specimen | Female (%) | Male (%) | Total (%) |
|---|---|---|---|
| Urine | 45 (62.5) | 27 (37.5) | 72 (51.4) |
| Sputum | 10 (62.5) | 6 (37.5) | 16 (11.4) |
| High vaginal swab | 14 (100) | - | 14 (10.0) |
| Urethral swab | - | 15 (100) | 15 (11.0) |
| Ear swab | 3 (33.3) | 6 (66.7) | 9 (6.4) |
| Wound swab | 4 (28.6) | 10(71.4) | 14 (10.0) |
| Total | 76 (54.3) | 64 (45.7) | 140 |

**Table 4** Age Distribution of bacterial isolates-(Males %)

| Age Range | *P. mirabilis* (%) | *E. coli* (%) | *P. aeruginosa* (%) | *K. pneumonia* (%) | *S. aureus* (%) | Total (%) |
|---|---|---|---|---|---|---|
| <20 | - | 4 (80.0) | - | 1 (20.0) | - | 5(12.5) |
| 21-30 | 5 (28.0) | 5 (28.0) | 2 (11.1) | 1 (6.0) | 5 (28.0) | 18(45.0) |
| 31-40 | 2 (25.0) | - | - | 3 (37.5) | 3 (37.5) | 8(20.0) |
| 41-50 | 1 (17.0) | 1(17.0) | - | 1 (17.0) | 3 (50.0) | 6(15.0) |
| 51-60 | - | - | 1 (33.3) | - | 2 (67.0) | 3(7.5) |
| Total (%) | 8 (20.0) | 10(25.0) | 3 (7.5) | 6 (15.0) | 13 (32.5) | 40 |

**Table 5** Age Distribution of bacterial isolates (Female %)

| Age Range | *P. mirabilis* (%) | *E. coli* (%) | *P. aeruginosa* (%) | *K. pneumonia* (%) | *S. aureus* (%) | Total (%) |
|---|---|---|---|---|---|---|
| <20 | 1 (14.3) | 5 (71.4) | 1 (14.3) | - | - | 7 (17.1) |
| 21-30 | 4 (25.0) | 6 (37.5) | 2 (12.5) | 2 (12.5) | 2 (12.5) | 16 (39.0) |
| 31-40 | 3 (25.0) | - | 2 (17.0) | 1 (8.3) | 6 (50.5) | 12 (29.1) |
| 41-50 | 1 (33.3) | - | - | - | 2 (67.0) | 3 (7.3) |
| 51-60 | - | - | - | 1 (50.0) | 1 (50.0) | 2 (4.9) |
| >60 | - | 1 (100) | - | - | - | 1 (2.4) |
| Total (%) | 9 (22.0) | 12 (29.3) | 5 (12.2) | 4 (10.0) | 11 (27.0) | 41 |

**Table 6** Distribution of bacterial isolates by specimen

| Specimen | *P. mirabilis* (%) | *E. coli* (%) | *P. aeruginosa* (%) | *K. pneumonia* (%) | *S. aureus* (%) | Total (%) |
|---|---|---|---|---|---|---|
| Urine | 13 (21.3) | 18 (29.5) | 4 (6.5) | 7 (11.5) | 19 (31.1) | 61 (75.3) |
| Sputum | 1 (100) | - | - | - | - | 1 (1.2) |
| High vaginal swab | 1 (25.0) | 2 (50.0) | - | 1 (25.0) | - | 4 (4.9) |
| Urethral swab | 2 (100) | - | - | - | - | 2 (2.5) |
| Ear swab | - | 1 (16.7) | 2 (33.3) | - | 3 (50.0) | 6 (7.4) |
| Wound swab | - | 1 (14.3) | 2 (28.6) | 2 (28.6) | 2 (28.6) | 7 (8.6) |
| Total | 17 (20.9) | 22 (27.2) | 8 (10.0) | 10 (12.3) | 24(29.6) | 81 |

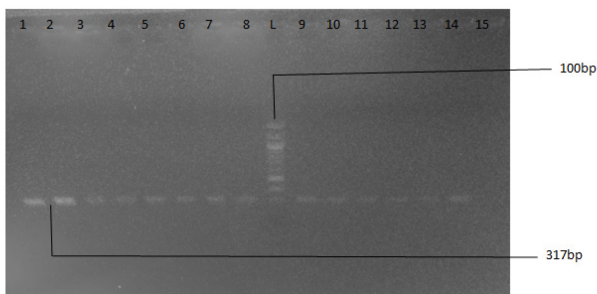


**Plate 1** Agarose Gel Electrophoresis of the UreC gene of some selected bacterial isolates. Lane 1-14 represents the UreC gene bands (317bp). Lane L represents the 100bp Molecular ladder.

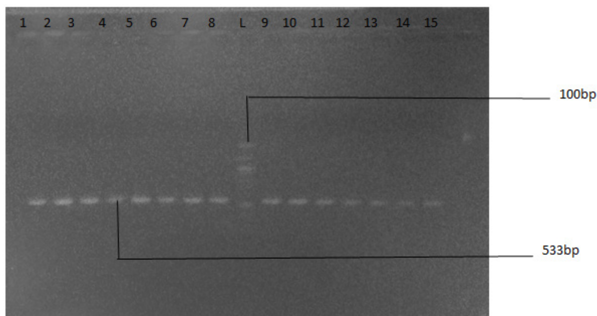


**Plate 2** Agarose Gel Electrophoresis showing bands of ZapA gene (533bp). Lane 1–15 represents the samples while L represents 100bp molecular ladder.

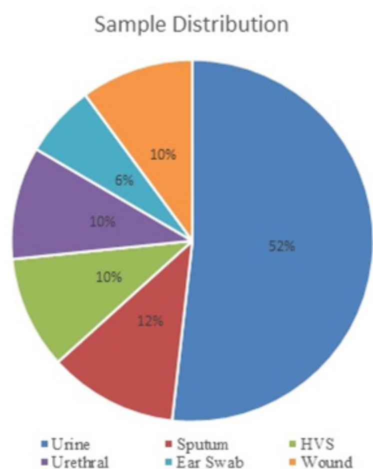


**Figure 1** Pie chart showing the distribution of clinical samples.

## Discussion

*P. mirabilis* uses various set of virulence factors to access and colonize the host urinary tract, including urease and protease. It accounts for most of the urinary tract infections that occur in hospital settings and for 90% of *Proteus* infections.[15]

A sum total of one-hundred and forty (140) samples were collected; 76(54.3%) were from female patients and 64(45.7%) were from male patients. Of the 72(51.4%) urine specimens, 27(37.5%) were obtained from males while 45(62.5%) were from females. Of the 16(11.4%) sputum samples, 10(62.5%) were obtained from female patients while 6(37.5%) were from males. A total of 14(10.0%) High vaginal swabs (HVS) were collected from 14(100%) female patients. A total of 15(11.0%) urethral swabs were obtained from 15(100%) male patients. Of the 9(6.4%) ear swabs, 6(66.7%) were from males while 3(33.3%) were from females. Of the 14(10.0%) wound swabs, 10(71.4%) of the samples were obtained from male patients while 4(28.6%) were from female patients.

In male patients below 21 years of age, 5(12.5%) isolates were recovered, 4(80%) of which were *E. coli* and 1(20%) was *K. pneumonia*. In males within the age range of 21-30 years, 18(45.0) isolates were recovered; 5(28.0%) were *E. coli*, 2(11.1%) were *P. aeruginosa*, 1(6.0%) was *K. pneumonia* and 5(28.0%) were *S. aureus*. A total of 8(20.0%) isolates were recovered from male patients within the age range of 31-40 years; 2(25.0%) were *P. mirabilis*, 3(37.5%) were *K. pneumonia* and 3(37.5%) were *S. aureus*. In males within the age of 41-50 years, 6(15.0%) isolates were recovered, 1(17.0%) was *P. mirabilis*, 1(17.0%) was *E. coli*, 1(17.0%) *P. aeruginosa* and 3(50.0%) were *S. aureus*. Isolates recovered from male patients within the age range of 51-60 years were 3(7.5%), of which 1(33.3%) was *P. aeruginosa* and 2(67.0%) were *S. aureus*.

For female patients below the age of 21 years old, 7(17.1%) isolates were recovered; 1(14.3%) *P. mirabilis*, 1(14.3%) *P. aeruginosa* and 5(71.4) *E. coli*. Within the age range of 21-30 years, 16(39.0%) isolates were recovered; 4(25.0%) *P. mirabilis*, 6(37.5%) *E. coli*, 2(12.5%) *P. aeruginosa*, 2(12.5%) *K. pneumonia* and 2(12.5%) *S. aureus*. In females within the ages of 31-40 years, the total isolates recovered were 12(29.1%), of which 2(25.0%) were *P. mirabilis*, 2(17.0%) were *P. aeruginosa*, 1(8.3%) was *K. pneumonia* and 6(50.5%) were *S. aureus*. A total of 3(7.3%) isolates were recovered from females within the age range of 41-50 years; 1(33.3%) *P. mirabilis* and 2(67.0%) *S. aureus*. For females within 51-60 years old, 2(4.9%) isolates were recovered; 1(50.0%) *K. pneumonia* and 1(50.0%) *S. aureus*. Only 1(2.4%) isolate was recovered from females above 60 years old and that was 1(100%) *E. coli*. In summary, a total of 8(20.0%) and 9(22.0%) *P. mirabilis* were recovered from male and female patients respectively. A total

of 10(25.0%) and 12(29.3%) *E. coli* were from males and females respectively. A total of 3(7.5%) *P. aeruginosa* were recovered from male patients and 5(12.2%) were from females. A total of 6(15.0%) *K. pneumonia* were recovered from males and 4(10.0%) were from females. A total of 13(32.5%) *S. aureus* were recovered from male patients and 11(27.0%) were from females.

A total of eighty-one (81) isolates were recovered. A total of 61(75.3%) out of 72 urine specimens yielded 13(21.3%) *P. mirabilis*, 18(29.5%) *E. coli*, 4(6.5%) *P. aeruginosa*, 7(11.5%) *K. pneumonia* and 19(31.1%) *S. aureus*. Out of the 16 sputum samples, only 1(1.2%) yielded 1(100%) *P. mirabilis.* A total of 4(4.9%) HVS out of 14 yielded 1(25.0%) *P. mirabilis*, 2(50.0%) *E. coli* and 1(25.0%) *K. pneumonia*. A total of 2(2.5%) urethral swabs out of 15 yielded 2(100%) *P. mirabilis*. A total of 6(7.4%) ear swabs out of 9 yielded 1(16.7%) *E. coli*, 2(33.3%) *P. aeruginosa* and 3(50.0%) *S. aureus*. A total of 7(8.6%) wound swabs out of 14 yielded 1(14.3%) *E. coli*, 2(28.6%) *K. pneumonia* and 2(28.6%) *S. aureus*. In summary, a total of 17(20.9%) *P. mirabilis*, 22(27.2%) *E. coli*, 8(10.0%) *P. aeruginosa*, 10(12.3%) *K. pneumonia* and 24(29.6%) were recovered.

Of the 17 isolates of *P. mirabilis*, 10(58.8), 8(47.1), 13(76.5), 11(64.7), 17(100), 17(100), 17(100), 16(94.0), 16(94.0) and 17(100), were sensitive to Tarivid, Reflacine, Ciprofloxacin, Augmentin, Gentamycin, Streptomycin, Ceporex, Nalidixic acid, Septrin, and Ampicillin respectively. Plates 1 and 2 depict agarose gel electrophoresis of *ZapA* and *UreC* gene respectively. Fourteen (14) of 15 samples subjected to PCR amplification were positive for the *UreC* gene at 317bp while all the 15 were positive to *ZapA* gene at 533bp. Lane L is the 1000bp Quick-Molecular ladder. Out of the 140 clinical specimens collected, 76(54.3%) were from females while 64(45.7%) were from males. This indicates that the frequency of hospital attendance is higher for females than males. This analysis therefore agrees with Bertakis *et al.,* 2000 whose study showed that females are more likely to attend health clinics and utilizes health care services than the male counterparts.

Individuals within the age range of 21-30, are prone to any sexually transmitted infections due to their active sexual lifestyle with multi-sex partners as well as physical activities. The present study reveals that the highest number of *P. mirabilis* were isolated in males 5(28.0%) and females 4(25.0%) within these age range (21-30years). This is in agreement with the Center for Disease Control and Prevention (CDCP, 2015)[16] which stated that regardless of race or gender, sexually active adolescents and young adults between 15-30 years are at increased risk for STIs when compared to older adults. It also counters the statement of Cheesbroug,[7] which states that *P. mirabilis* is a common cause of urinary infection in the elderly and young males and often following catheterization or cystoscopy.

Armbruster et al.,[10] in his study posited that females are mostly affected by urinary tract infections than males. The high incidence of UTIs in females could be as a result of sexual activity, shorter urethra, birth control or menopause.[7] Our present study was in tandem with their study as the total number of *P. mirabilis* isolated from different clinical specimens were 17 with slightly more isolates from females 9(22.0%) than males 8(20.0%).

It has been hypothesized that *P. mirabilis* has access to the bladder by infecting the periurethral area. Apart from these, once an infection is established, the pathogen passes through the urethra via swarming motility to the bladder. *P. mirabilis* binds to bladder epithelial cells where it eventually colonizes.[17] In this study, the incidence of *P. mirabilis* in relation to the clinical specimens revealed that the most frequent occurrence was seen in urine {13(21.3%)} and this coincides with the study of Agbagwa and Ifeanacho (2005) which indicated that pathogens causing urinary tract infections (UTIs) are mostly isolated from urine specimens. As an opportunistic pathogen, it was also isolated from sputum, high vaginal swab (HVS) and urethral swab. The antibiotic susceptibility pattern showed that *P. mirabilis* is susceptible to the Ciprofloxacin, Gentamycin, Streptomycin, Ceporex, Nalidixic acid, Septrin and Ampicillin. This is in agreement with Cheesbrough,[7] in quote, "antibiotics with activity against *P. mirabilis* include ampicillin, cephalosporins and aminoglycosides". The result was also in agreement with Tsai et al.[18]

The results of the agarose gel electrophoresis revealed 533bp for *ZapA* gene and 317bp for *UreC* gene with complete positive bands which was in accordance with the study carried out by Ali and Yousif 2015. *UreC* gene is responsible for the production of urease enzyme which causes the elevation of urine pH, resulting in stone formation and other UTIs.[19] Dumanski et al.,[20] reported that urease during *Proteus* UTI is the accumulation of toxic levels of ammonia from urease-mediated hydrolysis of urea that damages tissues including renal epithelia. *ZapA* is a metalloprotease capable of mediating the degradation of numerous host proteins *in vitro.* According to Belas et al.,[21] protease enzyme is capable of cleaving IgA, IgG, secretory component and antimicrobial peptides which reduces their antimicrobial activity. *ZapA* may also contribute to evading the innate immune response during infection of *P. mirabilis.* Therefore, the presence of these genes makes the organism more pathogenic and resistant to antibiotics curing effect.

## Conclusion

With the aid of Polymerase Chain Reaction (PCR) molecular technique, we have been able to detect the high presence of *UreC* and *ZapA* virulent genes in *Proteus mirabilis* isolated from different clinical specimens in our hospitals in Bayelsa state; which makes them more pathogenic and resistant to antibiotics curing effect. Therefore, PCR and other molecular diagnostic methods hold the hope of making rapid diagnosis and directed therapy a reality.

### What is known about this topic?

- Naturally the body flourishes from co-existing with trillion of bacteria, bacteria in turn become defensive by developing resistant genes in form of *UreC* and *ZapA* central to assault by chemicals supposedly designed to killed them.
- These resistant genes in *Proteus mirabilis* are common in the hospital environment and can easily be transmitted.

### What this study adds

- The molecular identification of these two genes associated with *P. mirabilis* was carried out with high through put technology in the South-South region of Nigeria.
- People are now increasingly becoming aware of the resistant genes that can easily be transmitted from *P. mirabilis* in the hospital environments in the South-South region of Nigeria
- The study showed that what keeps us healthy is our immune system, and that proper hygiene and sanitation is very much high yield in living healthy.

## Authors' contributions

Alade Tolutope: Conception and design; Itodo, Sunday Ewaoche: manuscript drafting, revising manuscript for intellectual content and data analysis; Arikekpar Ibemologi: Conception and design; Ekanem Edmund: Data collection and statistical analysis. The authors read and agreed on the final version of the manuscript.

## Acknowledgments

We wish to wholeheartedly express our profound gratitude to Prof. Tatfeng Y.M for tutoring, professional advice and for availing us the opportunity to make use of his Molecular Laboratory.

## Competing interest

The authors declared no competing interest.

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