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REGULAR ARTICLE

ANTIBACTERIAL SURVEILLANCE: AN APPROACH TO MITIGATING MULTI-DRUG RESISTANCE MENACE AMONG CLINICAL UROPATHOGENS IN EKITI STATE, NIGERIA

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ABSTRACT

Urinary tract infections (UTIs) are among the most common human infections with the distribution of etiological agents and antibiotic resistance patterns varying from region to region and from time to time. The aims of this study were to ascertain the prevalence and antibiotic resistance profiles of common Gram-negative uropathogens among patients attending a Tertiary Care Hospital in Ekiti State, Nigeria. One hundred and fifty clean-catch midstream urine specimens were obtained and cultured within 2 hours of collection for the detection of Gram-negative uropathogens. The isolated organisms were identified by standard microbiological methods. Of the total 150 urine specimens analyzed, 82 (54.67%) specimens were positive for Gram-negative uropathogen with significant bacteriuria of which 34 (41.46%) were males and 48 (58.54%) were females. Klebsiella spp. 38 (46.34%) and Escherichia coli 32 (39.02%) were the most frequently isolated Gram-negative uropathogens, followed by Proteus mirabilis 10 (12.20%) while the least occurring uropathogen was observed to be Pseudomonas aeruginosa 2 (2.4%). All the isolated uropathogens were observed to be highly resistant to the commonly prescribed antibiotics. Emerging resistance to carbapenems was also observed. Nevertheless, carbapenems showed highest susceptibility compared to other tested antibiotics. Conclusively, high levels of resistance of uropathogens to antibiotics exist in our setting. This therefore calls for continuous antibiotic surveillance and improved antibiotic stewardship.

Keywords: Gram-negative uropathogens, UTIs, Antibiotic resistance, Klebsiella spp., Escherichia coli

INTRODUCTION

Urinary tract infections (UTIs) are among the most common human infections existing in our setting. This therefore calls for continuous antibiotic surveillance and improved antibiotic stewardship.

Sample collection

Urine specimens were collected in accordance to the standardised protocols as described by Cheesbrough (2006) and modified by Prakash and Saxena (2013) and Ochada et al. (2014). Clean catch midstream urine (MSU) was collected from each patient into a 20ml calibrated sterile screw-capped universal container which was distributed to the patients. All patients were well instructed on how to collect sample aseptically prior to sample collection to avoid contaminations from urethra. Samples collected were transported to the laboratory in ice pack and cultured within 2 hours of collection for Gram-negative uropathogen isolation.

Culture and Isolation of Organism

The urine specimens (10 μl) were cultured on Cysteine Lactose Deficient agar (CLED), MacConkey, and Eosin Methylene blue (EMB) agar simultaneously, using micropipette and incubated in aerobic conditions for 24 hours at 37°C. Cultures without any colony at the end of 24hrs of incubation were further incubated for 48hrs. Plates with colony count equal to or more than 10^5CFU/ml were considered significant culture positive (Pooja et al., 2017). The organisms were further subcultured on fresh MacConkey agar plate in order to get pure culture.

Identification of Isolates

The isolates were identified and confirmed using standard biochemical tests as described by Barrow and Feltham (2003) and stored on nutrient agar slants for further study.

Antibiotic Susceptibility Assay

The antibiotic susceptibility testing was performed according to CLSI guidelines (CLSI document (M100-S21), 2015). The antibiotic discs and their concentrations consisted of ceftriaxone (CRO, 30μg), ceftazidime (CAZ, 30μg), gentamicin (CN, 10μg), ampicillin (AM, 10μg), norfloxacin (NOR, 10μg), tetracycline (TE, 30 μg), erythromycin (ETP, 10 μg) and meropenem (MEM, 10 μg). Moreover, prior to antibiotic susceptibility testing, the culture was diluted in sterile normal saline (0.9%/v) suspension and thereafter matched with the 0.5 McFarland standard (Ojo et al., 2013).
Ethical Approval

Ethical approval with protocol number (ERC/2017/10/18/84B) was obtained before conducting the research. All research protocols were performed in accordance to the ethical standards of committees on human experimentation laid down in Helsinki declaration of 1964 revised in 2000 (World Medical Association Declaration of Helsinki, 2000).

RESULTS

A total of 150 urine specimens recommended for Urine Microscopy, Culture and Sensitivity at a Tertiary Care Hospital in Ekiti State, Nigeria were collected and analyzed. Eighty-three (55.33%) urine specimens were collected from females while 67 (44.67%) urine specimens were collected from males. Urine specimens of only 34 (41.46%) males and 48 (58.54%) females showed demonstrable bacteruria and they were all positive for Gram-negative uropathogens. Out of the 82 positive samples, 57 (69.51%) urine specimens were from outpatients while 25 (30.49%) urine specimens were from inpatients (Table 1).

| Table 1 Percentage of Patients’ Demographic Information | Frequency n (%) |
|----------------------------------------------------------|-----------------|
| Patient Demographic Characteristics                     |                 |
| Sex                                                      |                 |
| Positive samples                                         |                 |
| Male                                                     | 34 (41.46)      |
| Female                                                   | 48 (58.54)      |
| Total                                                    | 82 (54.67)      |
| Negative samples                                         |                 |
| Male                                                     | 33 (48.53)      |
| Female                                                   | 35 (51.47)      |
| Total                                                    | 68 (45.33)      |
| Location                                                 |                 |
| Positive samples                                         |                 |
| Outpatient                                               | 57 (69.51)      |
| Inpatient                                                | 25 (30.49)      |
| Total                                                    | 82 (54.67)      |
| Negative samples                                         |                 |
| Outpatient                                               | 40 (58.82)      |
| Inpatients                                               | 28 (41.18)      |
| Total                                                    | 68 (45.33)      |
| Total samples collected                                  | 150             |

The uropathogens were more isolated from the test subjects between the age brackets 11-40 years and >70 years. Males were mostly affected in their late ages above 70 while females were mostly affected in their reproductive age group (11-40 years) (Table 2).

| Table 2 Age-group and Frequency of Patient with Significant Bacteruria | Frequency n (%) |
|-----------------------------------------------------------------------|-----------------|
| Age group                                                             | Male | Female | Total |
| ≤10                                                                   | 1 (100) | 0 (0) | 1 (4) |
| 11-40                                                                 | 4 (33.33) | 8 (66.67) | 12 (48) |
| 41-70                                                                 | 2 (50) | 2 (50) | 4 (16) |
| >70                                                                   | 5 (62.50) | 3 (37.50) | 8 (32) |
| Total                                                                 | 12 (48) | 13 (52) | 25 (100) |

Of the total 150 urine specimens collected, 97 (64.67%) urine specimens were from outpatients while 53 (35.33%) were from inpatients. Most of the urine specimens were from Obstetrics and Gynaecology Department 29 (19.33%), General Outpatient Department 23 (15.33%), Accident and Emergency 17 (11.33%), Urology 15 (10%), Female Medical Ward 14 (9.33%) and Surgical Outpatient Department 13 (8.67%), (Fig. 1).

The overall resistance rates of all the bacterial isolates to ampicillin, gentamicin, tetracycline, ceftriaxone, ceftazidime, ertapenem, meropenem and norfloxacin were observed to be 93.90%, 87.80%, 78.05%, 60.98%, 71.95%, 41.46%, 28.05% and 68.29% respectively (Table 3).
DISCUSSION

The present results showed that Klebsiella spp. and E. coli were the most frequently isolated uropathogens. This finding is in agreement with that of Abugwa and Ieanaacho (2015) in Rivers State, Nigeria, where Klebsiella spp. and E. coli were the most isolated Gram-negative uropathogens. However, this contradicts the reports in other studies, where Pseudomonas aeruginosa (Ehinmidu, 2003) and Staphylococcus aureus (Ekwealor et al., 2016) were observed as the most sensitive drug, followed by ertapenem and these are not drugs often used and misuse of antibiotics and the consumption of substandard antibiotics as increases with increasing age for both sexes (Ani and Mgbechi, 2008; Nicolle, 2011). Urine specimens were more obtained from outpatients 94 (77%) than inpatients 28 (23%), meaning that most cases were coming in directly from the community. This is in agreement with the studies from Nigeria (Iregbu and Nwajiobi-Princewill, 2013), Botswana (Renuart, 2013) and the United States (Doyle et al., 2001). The high prevalence of UTI recorded among patients in Obstetrics and Gynaecology department in the present study agrees with the findings of Devi and Rajkumar (2012), who reported that the risk of UTI is most prevalent among patients with gynaecological problems. In the present study, majority of the isolates showed resistance to commonly employed drugs in the treatment of UTIs. However, meropenem was the most sensitive drug, followed by ertapenem and these are not drugs often deployed as first line of treatment of uncomplicated UTI. This is in agreement with the study carried out by Iregbu and Nwajiobi-Princewill (2013) who reported similar antibiotic resistance patterns. However, this contradicts the findings in various studies from different part of the world where resistant rates reported were different (Ehinmidu, 2003; Raza et al., 2011). The indiscriminate use and misuse of antibiotics and the consumption of substandard antibiotics as earlier reported by Okeke and Lamikanra (1995) might have over the years contributed to the high rate of antibiotic resistance observed in the present study.

CONCLUSION

The results indicated that Klebsiella spp. and Escherichia coli isolates are the most common cause of UTI in our environment. All the isolates showed high resistance to commonly isolated uropathogens (like ceftazidime and ceftriaxone), gentamicin, tetracycline and norfloxacin were observed to be high. The findings also revealed that E. coli isolates were more susceptible to meropenem and ertapenem than Klebsiella spp. isolates (Table 4).

| Table 3 Overall antibiotic susceptibility and resistance of the isolated Gram-negative uropathogens |
|----------------------------------------|
| Antibiotics | AMP | CN | TET | NOR | CFTX | CFTZ | ERTP | MERP |
|-------------|-----|----|-----|-----|------|------|------|------|
| Sus n (%)   | 5   | 61 | 10  | 12.20 | 18  | 21.95 | 26   | 31.71 |
| Res n (%)   | 77  | 93.9 | 72  | 87.80 | 64  | 78.05 | 56   | 68.29 |

**Keys:** Sus = Susceptibility, Res = Resistance, AMP = Ampicillin, CN = Gentamicin, TET = Tetracycline, NOR = Norfloxacin, CFTX = Ceftriaxone, CFTZ = Ceftazidime, ERTP = Ertapenem, MERP = Meropenem

High resistance rate to ceftazidime, ceftriaxone, norfloxacin, gentamicin, tetracycline and ampicillin were observed in 71.08%, 76.32%, 65.79%, 81.58%, 76.32% and 94.74% of the Klebsiella spp. isolates respectively. However, low resistance rate to meropenem and ertapenem was observed in 34.21% and 47.37% respectively. The rates of resistance among all the Gram-negative uropathogens to ampicillin, third generation cephalosporins (like ceftazidime and ceftriaxone), gentamicin, tetracycline and norfloxacin were observed to be high. The findings also revealed that E. coli isolates were more susceptible to meropenem and ertapenem than Klebsiella spp. isolates (Table 4).

| Table 4 Antibiotic resistance of the isolated Gram-negative uropathogens |
|----------------------------------------|
| Antibiotics | Isolated Uropathogens | No. | AMP | CN | TET | NOR | CFTX | CFTZ | ERTP | MERP |
|-------------|----------------------|-----|-----|----|-----|-----|------|------|------|------|------|
| Klebsiella spp. | 38 | 94.74% | 81.58% | 76.32% | 65.79% | 76.32% | 71.05% | 47.37% | 34.21% |
| E. coli | 32 | 100% | 87.50% | 100% | 87.50% | 50% | 50% | 50% | 50% |
| P. mirabilis | 10 | 80% | 80% | 70% | 50% | 50% | 50% | 50% |
| P. aeruginosa | 2 | 100% | 100% | 50% | 100% | 100% | 50% | 50% |

**Keys:** Klebsiella spp. = Klebsiella species, E. coli = Escherichia coli, P. mirabilis = Proteus mirabilis, P. aeruginosa = Pseudomonas aeruginosa, AMP = Ampicillin, CN = Gentamicin, TET = Tetracycline, NOR = Norfloxacin, CFTX = Ceftriaxone, CFTZ = Ceftazidime, ERTP = Ertapenem, MERP = Meropenem

The incidence of UTI among female patients compared to male patients in the present study could be attributed to the physiological and anatomical differences in males and females (Abugwa and Ieanaacho, 2015). However, the reduction of UTI in males may be attributed to the longer distance between the anus and urethra meatus and the dry environment in the urethra of males which reduces microbial growth (Prakash et al., 2012; Kibret and Aberra, 2014). This finding agrees with the report of Abugwa and Ieanaacho (2015) in Rivers State, Nigeria, where the indiscriminate use and misuse of antibiotics and the consumption of substandard antibiotics as earlier reported by Okeke and Lamikanra (1995) might have over the years contributed to the high rate of antibiotic resistance observed in the present study.

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