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## Original article

# Detection of colistin resistant Gram-negative bacteria in hospital cleaning Staff with asymptomatic bacteriuria; implications for community spread.

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**ABSTRACT**

**Background:** Asymptomatic bacteriuria (ASB) is frequently found in many healthy people of both sex and all ages and the indiscriminate use of antibiotics in its treatment has been recognized as a major contributor to the emergence of antibiotic resistance. The emergence of colistin resistance, an antibiotic used as the last resort in apparently healthy individuals, is a threat to global public health. **Aim:** The study was undertaken to determine the prevalence of asymptomatic bacteriuria (ASB) in hospital cleaning staff (HCS) and colistin resistance in *Escherichia coli*, and *Pseudomonas aeruginosa.* **Methods:** A total number of 80 midstream urine specimens were obtained from HCS, each cultured and isolates of *E. coli* and *P. aeruginosa* identified based on standard biochemical procedures. The sensitivity to colistin was determined by the colistin broth disk elution (CBDE) method. **Results:** The ASB prevalence was 27.5% with *E. coli* and *P. aeruginosa* having a prevalence of 54.5% and 45.5% respectively. Colistin resistance was observed in 13.6% *E. coli and* 36.6% of *P. aeruginosa* isolates. **Conclusion:** The presence of colistin resistance amongst *E. coli* and *P. aeruginosa* isolates of HCS should be seen as an imminent threat that warrants prompt action. .

## Introduction

There has been a steady increase in antibiotic resistance among multidrug-resistance Gram-negative bacteria globally [1]. This has presented a serious health challenge as the therapeutic options for managing infections caused by them have narrowed [2]. As a result of this rapid evolution of antimicrobial resistances among bacteria to all the different classes of antibiotics available in the market, coupled with lack of interest among the pharmaceutical companies in funding research and development of new antimicrobial drugs attention has shifted to old antimicrobial agents that have been abandoned because of their presumed toxicity [3]. The global challenge of antimicrobial resistance especially in the treatment of multidrug-resistant (MDR) Gram-negative bacteria coupled with the lack of availability of new classes of drugs remains quite critical [3]. Due to the high multidrug-resistance among Gram-negative bacteria and the unavailability of new drugs to combat them, attention has turned to colistin, also known as polymyxin E, as the last-resort antibiotic for the management of MDR Gram-negative

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bacteria [1-4]. Colistin, first introduced in Japan in 1947 is a member of an old class of cationic, cyclic, polypeptide antibiotics [3]. The use of colistin was abandoned in the 1980s due to the concerns about its toxicity in the kidney and nerves, however, its intravenous injection as colistin sulphate has been shown to be considerably safe for the management of severe infections caused by sensitive Gram-negative pathogens [3]. Currently, colistin is the empiric drug for the management of infections caused by multidrug-resistant Gram-negative pathogens. However, in recent years there has been an unprecedented rise in resistance evolution in bacteria to colistin antibiotics thus limiting the therapeutic options available for infection management. This is creating an alarming public health concern requiring that concerted efforts should be made to limit this development through prudent and judicious use of the drug by the relevant health authorities and regulatory bodies.

Hospital cleaning staff play essential roles in the management of hospital routine activities and maintenance of the normal operations in the hospital, yet they received far less training on nosocomial infections awareness and management of nosocomial infections prevention. Moreso, there has been long-standing awareness that hospital cleaning staff (HCS) have great potentials to spread infection due to the number of patient rooms they enter within a shift [5], and because of their diverse and extensive daily duties, which may expose the patients to nosocomial infections [6]. They inadvertently become vehicles of infection transmission.

Asymptomatic bacteriuria (ASB) is defined as having urine specimen with an isolation of organism in quantitative counts of $\geq 10^5$ cfu/mL without symptoms of a urinary tract infection [7-9]. These symptoms are dysuria, urinary frequency or urgency, or suprapubic pain in patients with simple cystitis, and fevers with cystitis symptoms, flank pain, or costovertebral angle tenderness in patients with acute complicated urinary tract infections [7].

Asymptomatic bacteriuria is commonly misdiagnosed as urinary tract infection leading to an increasing and persistent rate of antibiotic therapy for ASB despite recommendations against it [10,11], Inappropriate use of antibiotics for ASB can result in adverse outcomes such as increased prevalence of antimicrobial resistance in urinary tracts organisms in the community as well as rapid colonization with multidrug resistant organisms [12]. Multidrug resistant bacteria implicated in UTIs include extended-spectrum beta-lactamase (ESBL) producing organisms [13-15], carbapenem-resistant *Enterobacteriaceae* (CRE), and colistin-resistant Gram-negative bacilli [10,16].

While the prevalence of ASB in healthy premenopausal and pregnant women and in long-term catheterized patients are well known [17, 18], so also the epidemiology and pathogenic mechanisms of uropathogenic *E. coli* have been extensively studied [19,20], data about ASB in HCS and pathogenesis of UTIs caused by other organisms like *P. aeruginosa* are limited in Nigeria. Data on the prevalence of ASB among HCS in rural hospitals where negligence is high and lack of supervision of HCW are expected to be laxed would help to determine generally their role in infection dissemination. Therefore, this study was aimed at ascertaining the prevalence of asymptomatic bacteriuria (ASB) and colistin resistance in Gram-negative bacteria amongst Hospital Cleaning Staff in sub-urban Hospitals in Delta State, Nigeria.

## Methods

### Ethics and informed consent

Ethical clearance was approved for the study by the Delta State University research ethics committee (ref no: SOE I011SOMO01) and permission to collect samples was obtained from the Delta State Department of Health (ref no: DC-20146RP10-487) while informed consent was obtained from patients before collecting samples from them and strict confidentiality was maintained.

### Study area and population.

This study was conducted to determine the prevalence of ASB among HCS in rural communities of Obiaruku, Umutu and Agbor. In terms of level of development, Umutu is the least developed followed by Obiaruku and then Agbor which is a semiurban location. The study was carried out from February to April 2017 and included apparently healthy HCS aged $\geq 18$ years. The hospitals were selected due to the large number of patients they handle on a regular basis as well as their locations which cover a wide geographical area of the state. The cleaning staff in the hospitals picked for this study perform duties that entail but not limited to the cleaning of patient room, other working staff offices, including mopping floors, scrubbing toilets, disinfecting surfaces, emptying wastes, and restocking cleaning and hygiene supplies. The HCS attend to patients admitted in both general wards and intensive care units (ICU). To apply inclusion and exclusion criteria, the HCS were simply interviewed and asked if they had fever ≥38°C and any other urinary tract infection (UTI) symptoms, persons with symptoms of UTIs were excluded. Voluntary participating HCS comprising of nursing assistants and cleaning staff were enrolled in the study. Absolute confidentialities were promised and maintained as no biodata of the participants except sex and age were collected. All samples were de-anonymised, and it was one-off sampling**.**

**Sample Collection and Identification**

A total of 80 midstream urine samples were collected into a sterile urine container from HCS attending to admitted patients in general wards and ICU of 3 hospitals located in rural communities of Delta State, Nigeria. 50 mL sterile Nalgene tubes were used in the sample collection and sampling was done aseptically. Immediately upon receipt of samples, they were transported in icepack to the Microbiology laboratory of the Delta State University, Abraka. The urine samples were cultured on MacConkey agar plates (Oxoid limited, United Kingdom) and incubated for 24 h at 37 °C, for the purpose of identifying two important pathogens: *E. coli* and *P. aeruginosa.* After overnight incubation, positive urine culture showing ≥100,000 colony-forming units (CFU)/mL of one or more bacteria were selected and sub-cultured. Lactose and non lactose fermenting colonies were picked and subjected to Gram staining. The non lactose fermenting colonies were inoculated on Triple Sugar Iron media (TSI). After overnight incubation, *Pseudomonas* species were identified when TSI medium showed no reaction in the butt and alkaline slant, oxidase test positive, citrate test positive, urease test negative, catalase test positive and indole test negative, motility test by hanging drop method showing motile bacteria. In addition to Gram stain and colonial morphology, *E. coli* isolates were identified by the indole, methyl red, Voges-Proskauer and citrate tests (IMViC tests) and by other methods previously described by Collee et al., [21]. The isolates obtained from the samples were stored at -20 ºC in glycerol stocks prepared from Muller Hinton broth after 24 hrs incubation at 37ºC.

**Colistin Disk Elution Test**

The isolates from positive cultures were tested for their susceptibility to colistin by the colistin broth disk elution (CBDE) as recommended by CLSI [22] guidelines. This method used four glass tubes, and 10 mL of Cation-adjusted Muller Hinton broth (HI-media) was added to each tube. The first tube was used as a growth control (no antibiotic disc was added). One disc of colistin sulphate (10 µg) (Oxoid) was added to the second tube. Two discs of colistin sulphate (10 µg) were added to the third tube, and four discs of colistin sulphate (10 µg) were added to the fourth tube. The tubes were incubated at room temperature for 30-45 minutes to elute colistin from the medium. Study isolates stored in glycerol stocks were resuscitated in Muller Hinton broth and used to prepare a 0.5 McFarland solution in normal saline, and after mixing properly, 50 µL inoculum was added to each tube. The test tubes were mixed thoroughly and incubated at 37 °C for 24 h. The colistin MIC results were interpreted as per CLSI [22]. For quality control, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used. The Minimum Inhibitory Concentration (MIC) breakpoints of colistin, as per the recommendations of CLSI [22], were used in the interpretation. Here, MIC was the lowest drug concentration at which visible growth was inhibited after incubation**.**

**Results**

Out of the 80 urine samples from HCS included in the study, 22 (27.5%) ASB showing ≥100,000 colony-forming units (CFU)/mL of bacteria were selected for further analyses. Among them, 19 positive isolates were obtained from women (23. 8%) while 3 (3.7%) were from men (Table 1), and the mean age was 36 years.

The distribution of *E. coli* and *P. aeruginosa* in the 3 sampling locations are shown in Table 2. The number of *E. coli* isolates which was 12 (54.5%) was more prevalent than *P. aeruginosa* that was 10 (45.5%). The organisms were mostly isolated from urine of HCS in Umutu 12 (54.5%) and least in Agbor 3 (13.6%).

Colistin resistance profile of the isolates showed that 13.6% of *E. coli and* 36.6% of *Pseudomonas aeruginosa* isolates were resistant to the antibiotic tested as shown in Table 3. Statistically, no significant association exists between hospital cleaning staff and the prevalence of *E. coli* and *P. aeruginosa* based on the Chi-Square test (**$\chi^2$= 1.7271; p = 0.42166; df = 2)**, Monte Carlo simulation (**p =** 0.4486) and Fisher's Exact Test (p = 0.46959). Specifically, $\chi^2$ **value of 0.42166** indicates that the probability of observing this level of association by chance is 42.2%, which is much higher than the typical significance level of 0.05 (5%). However, the weak association indicated by Cramer's V (0.13275) and Contingency Coefficient (0.1316) suggests that other factors, such as environmental contamination, hygiene practices, or exposure to contaminated surfaces, might be influencing the prevalence of these bacteria.

Colistin resistance profile of the isolates showed that 13.6% of *Escherichia coli and* 36.6% of *pseudomonas aeruginosa* isolates were resistant to the antibiotic tested as shown in Table 3. Statistically, the chi-squared test ($\chi^2$ = 3.18, *p* = 0.204) and Fisher's exact test (*p* = 0.272) indicate no statistically significant association between colistin resistance and the hospital source of bacterial isolates. The Monte Carlo simulation (*p* = 0.229) supports this conclusion. Cramer's V (0.260) and Contingency Coefficient (0.252) also suggest a weak association between the variables, implying that colistin resistance is relatively evenly distributed across the hospitals studied, with no hospital showing a significantly higher prevalence.

**Table 1.** Sex distribution of urine samples among Hospital cleaning staff.

| Source of urine sample | Number of asymptomatic bacteriuria (n=22) | Number with no bacteriuria (n=58) |
|---|---|---|
| Male | 3 (3.7%) | 7(8.75%) |
| Female | 19(23.8%) | 51(63.8%) |
| Total | 22(27.5%) | 58(72.5%) |

**Table 2.** Prevalence of *E. coli* and *P. aeruginosa* among Hospital cleaning staff.

| Location | Bacteria | | |
|---|---|---|---|
| | No. of *E. coli* isolates (%) | No. of *P. aeruginosa* isolates (%) | Total No. (%) |
| Umutu | 6(27.3) | 6(27.3) | 12(54.5) |
| Obiaruku | 4(18.2) | 3(13.6) | 7(31.8) |
| Agbor | 2(9.1) | 1(4.5) | 3(13.6) |
| Total | 12(54.5) | 10(45.5) | 22(27.5) |

**Table 3**. Prevalence of colistin resistance among the bacterial isolates from the different hospitals.

| Location | Colistin resistance *E. coli* (%) | Colistin resistance *P aeruginosa* (%) |
|---|---|---|
| Umutu | 1(4.5) | 4(18.2) |
| Obiaruku | 1(4.5) | 3(13.6) |
| Agbor | 1(4.5) | 1(4.5) |
| Total | 3(13.6) | 8(36.6) |

**Discussion**

Asymptomatic bacteriuria is frequently unnecessarily treated with antibiotics, and this has been identified among the factors involved in the emergence of multi resistant bacteria among hospitalized patients with very high health costs [12]. Of the 80-urine obtained from HCS included in this study, 19 culture positive urine were from women (23. 8)%) while 3 were from men (3.7%) (Table 1). Most of the participants were indigens of the sampling location and less educated. The loop sided gender divide is attributable to the nature of the work involved in hospital cleaning and perceptions of workers. The primary role of hospital cleaners is to undertake regular and predominantly manual cleaning of the patient's immediate environment, toilets, bathroom, etc. In a rural setting like the sampling locations, such cleaning job descriptions enumerated are left for the women folks. The job satisfaction for male cleaners is low and most male accept hospital cleaning job when it is the only job available to them as there are limited opportunities for other more remunerating jobs [23].

Asymptomatic bacteriuria varies greatly with age, sex, and is a common finding in many populations, particularly healthy pregnant women, and persons with underlying urologic abnormalities. The mean age of the participants was 36 years. Studies have shown that the prevalence of ASB increases with age [24,25]. Banda et al., [26] in a study carried out in North-central Nigeria recorded the highest ASB prevalence in 24-33 age groups. Similarly, Fowora et al., [25] reported that the highest prevalence of ASB in their study was in the 25–31 age groups. Research on the prevalence of ASB in hospital cleaning staff is lacking. The prevalence of ASB among HCS in the current study (27.5%) was high but in line with a study conducted among apparently healthy women in Lagos, Nigeria (27.8%) [24]. Most studies investigated ASB in pregnant women and results varied with lower prevalence, ranging from10.3 to 24.7% among pregnant women [24,25].

In the transmission of microorganisms from environmental surfaces to patients, hands of HCS can be a source of nosocomial pathogens and outbreaks of infections by direct contact with contaminated reservoirs and this can be especially troubling with *P. aeruginosa* in immunodeficient patients [19, 20]. This is because *P. aeruginosa* is one of the commonest Gram-negative bacteria that takes advantage of an individual's weakened immune status to cause infection by its tissue-damaging toxins. *P. aeruginosa* can infect any anatomical site such as urinary tracts and ultimately be found in urine. The prevalence of *P. aeruginosa in* this study was 45.5%. The lower prevalence of *P. aeruginosa* obtained in urine as compared to *E. coli* (54.5%) is similar to the prevalence figures reported in most studies [15,19,20], where *E. coli* has higher prevalence than *P. aeruginosa* and usually the most prevalent isolate in urine specimen, [15,19,29].

One of the important contributors to rise of antimicrobial resistance is the inappropriate use of antibiotics in the treatment of ASB as it promotes the emergence of antimicrobial resistance [8]. Finding high resistance in colistin, a drug regarded as a last-line antibiotic amongst hospital cleaning staff, should draw extensive attention to the medical staff, such as doctors and nurses as well as the hospital management. Reports of antibiotic resistance transfer [1,13,34] and colistin resistance dissemination transfer horizontally by conjugative plasmids or vertically by chromosomal mutation have been observed[15]. Colistin resistance was initially attributed to *mcr* mobile genetic element first detected in *E. coli* isolates from an intensive pig farm in China [34]. Colistin usage in farming remains very high in Southeast Asia as well in agriculture in some countries in Africa [33 34]. The prevalence of *mcr* gene in different environments in Africa could be attributed to unregulated use of antimicrobial agents including colistin in the management of human and animal diseases. In a study done by Portal et al., [33] on rectal swab samples collected from mothers and their neonates in Nigeria between 2015-2016, forty mother and seven neonatal swabs carried *mcr* genes. In a systematic review on the global prevalence and molecular epidemiology of *mcr*-mediated colistin resistance in *Escherichia coli* clinical isolates, Dadashi et al., [34], reported 66.72%, 25.495, 5.19%, 2.27% and 0.32% in Asian, Europe, America, Africa, and Oceania respectively. These findings are quite troubling and demand that urgent actions be taken to curb this global widespread bacterial resistance to colistin.

The prevalence of colistin resistance among the study isolates although not very high should be a source of concern as it is the drug of last resort for the management of multidrug resistant Gram-negative pathogens. Increasing use of colistin for treatment of infections caused by Gram-negative bacteria has led to the emergence of colistin resistance in several countries worldwide [34]. Although resistance to polymyxins is generally less than 10% in many parts of the world, it is higher in the Mediterranean and South-East Asia (Korea and Singapore), where colistin resistance rates are continually increasing [34]. The probability of rapid dissemination occurring within the community and fueled by HCS in a local setting as our sampling locations is equally quite high. HCS receives far less information in the management of nosocomial infections prevention compared to medical staff. Additionally, they serve as agents for the two main mechanisms through which infections can be transferred viz; directly by physical environment, or indirectly through cross transmission via hand contamination. It should be quite worrisome that 50% of urine of apparently healthy HCS were infected with colistin resistant *E. coli* and *P. aeruginosa* and this call for an urgent attention. Detecting colistin resistant Gram-negative pathogens from apparently healthy individual indicates a huge health implication as there are chances of spreading resistant bacteria within the community by theses healthy individuals. Colistin resistance is suggestive of a multi drug resistance or extensive drug resistance profile as observed by Abd El-Baky et al., [1]. Therefore, the emergence of colistin resistance and by extension multidrug-resistant (MDR) or extensively drug-resistant (XDR) or pan-drug-resistant (PDR) *E. coli* and *P. aeruginosa* is a significant public health problem that can lead to a failed antimicrobial therapy.

## Limitation

Firstly, this study was carried out among small population of HCS in 3 rural hospitals of Delta State covering a limited geographical region and may not reflect overall picture of ASB in HCS across the country. Secondly, this study only determined colistin resistance phenotypically. Molecular analysis on colistin resistance which could have given a better picture was not performed on the isolates. However, this does not diminish the relevance of the findings of the study. More studies covering a larger area and sample size are recommended in order to generate a more accurate picture of the colistin resistance landscape in the region and country in general.

## Conclusion

The presence of colistin resistance amongst *E. coli* and *P. aeruginosa* isolates from HCS is an imminent threat that warrants appropriate action by the relevant health authority. The indiscriminate and perverse abuse of antibiotics should be discouraged and proper sensitivity testing of pathogens should be ascertained prior to antibiotics prescription by medical personals and clinicians in order to mitigate this budding challenge. Equally relevant is the urgency for prompt actions in effecting proper infection control by healthcare personnels. The agricultural use of antibiotics in animal husbandry should be limited to certain classes of antimicrobial agents that are not employed in treating bacterial infections in human so as to reduce the spread of resistance genes through environmental sources in the community.

## Author Contributions

OS Egbule and BC Iweriebor conceptualized and conducted the research and wrote the manuscript; OS Egbule, OL Oyubu and BC Iweriebor reviewed and corrected the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

All data and materials used analyzed during the current study presented in the article.

## Ethics approval and consent to participate.

This study protocol was reviewed and approved by the faculty of science Ethical Review Board. Authorization was obtained from the hospital management prior to the commencement of the study while informed consent was obtained from study participants and their participation was voluntary and declared in accordance with seven WHO 2011 Standards.

## Consent for publication.

All authors agreed to publish the article.

## Competing interests

The authors declare that they have no competing interests

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