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Prevalence of Asymptomatic Bacteriuria in a Sub-population of Tertiary Female Students: Antimicrobial Resistance Patterns and Group-specific Risk Factors

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ABSTRACT

Asymptomatic bacteriuria (ASB) is a major risk factor for urinary tract infections in most-at-risk populations. This study determined ASB positivity, common uropathogens, antimicrobial resistance (AMR) and risk factors of ASB among tertiary female students. A cross-sectional study was conducted on randomly recruited resident female students (n = 156). After questionnaire completion, urine samples were collected on appointment basis and routinely analyzed. Dipstick, microscopy, and urine culture methods were used to establish ASB positivity. Disk diffusion method was used to determine AMR. From 156 urine samples, 15 (9.6%) tested positive for ASB (single organism count > 10^5/mL from two consecutively voided mid-stream urine). A total of 24 isolates comprising: coagulase negative Staphylococci (CoNS) 9 (37.5%), *Klebsiella pneumonia* 6 (25.0%), *Staphylococcus aureus* 5 (20.8%), *Escherichia coli* 2 (8.3%), and *Serratia marcescens* 2 (8.3%). All isolates exhibited 100% susceptibility to nitrofurantoin and gentamicin but 100% resistance to ampicillin. CoNS were 100% sensitive to all antibiotics except cefuroxime and ampicillin. *S. aureus* and *K. pneumonia* exhibited acquired multidrug resistance to antibiotics belonging to ≥ 5 chemical classes. ASB positivity was 9.6%; nitrofurantoin and gentamicin were the most effective antibiotics, and ASB may be associated with poor personal hygiene.

Key words: Antimicrobial resistance, Bacteriuria, Multidrug resistance, Uropathogens and Ghana.

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INTRODUCTION

Asymptomatic bacteriuria (ASB) is variously defined as detection of significant bacterial count (single organism ≥ 10^5/ml from voided mid-stream urine [men] or single organism ≥ 10^5/ml from two consecutively voided mid-stream urine [women]) from individuals who are asymptomatic for urinary tract infections (Nicolle et al., 2005; Schnarr and Smaill, 2008; Tadesse et al., 2014). It is estimated that one in three females of childbearing age may experience ASB (Duarte and Gonzalez, 2008) of either single or multiple etiology. ASB may remain asymptomatic for many years exposing such group of females to high risk of bacteremia and sepsis (Weissenbacher and Reisenberger, 1993), pyelonephritis (Sheffield and Cunningham, 2005) and speculatively risk of fertility disorders later in adult life. Several reports have indicated that females are more predisposed to ASB than

their male counterparts (Shakya et al., 2014; Sharma and Paul, 2012; Yeshitela et al., 2012) and this has been related in part to the anatomy of the female genitourinary system (Boye et al., 2012; Feitosa et al., 2009) as well as hormonal, and some behavioral factors (Griebling, 2005).

As a result, females bear most of the ASB-related disease complications such as pyelonephritis and renal scarring (Raz, 2003). Many epidemiological studies from different geographical settings as well as in different female risk groups have associated sexual activity, number of sexual partners (Foxman and Chi, 1990; Vincent et al., 2013), post-coital voiding (Foxman and Chi, 1990), use of diaphragm (Fihn et al., 1996; Foxman and Chi, 1990), use of deodorant sanitary napkins (Foxman et al., 1995), use of spermicide-coated condoms (Fihn et al., 1998; Fihn et al., 1996), drinking caffeinated beverages (Foxman and Chi, 1990), and carbonated soft drinks (Foxman et al., 1995) to the risk of ASB and its attendant disease complications. Race has also been associated with ASB, for instance a study reported that colored women are five times more likely to contract ASB after first time sexual encounter than white women (Foxman et al., 1995).

It has been reported that drinking cranberry juice tends to protect women against ASB (Foxman et al., 1995). Many studies have provided varying reports regarding ASB prevalence, and antimicrobial resistance (AMR) patterns among most-at-risk (MAR) groups (Diamond et al., 1981; Gillespie et al., 1978; Oner et al., 2004), while other reports are divided on the most implicated uropathogen in ASB. Example, *E. coli* (Oner et al., 2004; Shakya et al., 2014; Sharma and Paul, 2012; Turpin et al., 2007; Vincent et al., 2013), coagulase negative Staphylococci (CoNS) (Bissong et al., 2013; Tadesse et al., 2014; Vincent et al., 2013), and *Staphylococcus spp* (Bissong, 2013; Turpin et al., 2007) have in separate studies been shown to be the most significant uropathogens involved in ASB. Of note, AMR, a byproduct of high rate of mutations in pathogens (mostly bacteria), use of sub-standard antimicrobial agents, and misuse of antibiotics has recently emerged as one of the greatest threats to treatment of infectious diseases. Meanwhile, knowledge of ASB prevalence trends and AMR patterns of implicated uropathogens in MAR populations are indispensable for prevention and treatment of urinary tract infections (UTIs) and AMR surveillance in resource-poor settings (RPSs).

In Ghana, the coverage, in terms of research data on ASB, UTIs and infectious diseases in general have disproportionately focused on children (Acquah et al., 2013), pregnant and menopausal women (Labi et al., 2015; Turpin et al., 2007), as well as diabetics. However it has neglected the female students in tertiary institutions who by logic are the potential mothers for future children. Meanwhile, the major challenge to ASB prevention, treatment and AMR surveillance among MAR groups such as female tertiary students in RPS has been lack of reliable data for health care planning and implementation. This study investigated ASB positivity, most common underlying uropathogens and their antibiotic susceptibility patterns, as well as host behavioral risk factors of ASB in a subpopulation of resident tertiary female students.

MATERIALS AND METHODS

Study Setting

The study was done at the University of Cape Coast (UCC), Cape Coast, Ghana. UCC is located in the Central Region of Ghana. It is the third largest public university in Ghana with a total student population of 59,834. It has six major halls of residence comprising four mixed halls (Oguaa Hall, Kwame Nkrumah Hall, VALCO Hall and Atlantic Hall), a male hall (Kingsley Hayford Hall) and a female hall (Adehye Hall).

Inclusion Criteria

Available female students of all year groups who at the time of sampling met the inclusion criteria: (1) a resident female student, (2) no complain of symptoms of urinary tract infections, (3) no antibiotic use for the past one month, and (4) have agreed by way of a signed written consent to partake in the study, were purposively recruited.

Study Design

A randomized cross-sectional study over a period of four months (January, 2013-April, 2013) was conducted to estimate prevalence of ASB, common uropathogens associated with ASB, AMR patterns, and group-specific risk factors of ASB among female students of UCC.

Sample Size

A total of 156 female students were recruited from five halls of residence excluding drop outs (that is, female students who completed the structured questionnaire but failed to return their urine samples on two consecutive appointment days and those who did not return both questionnaire and urine samples).

Ethical Consideration

Prior to the commencement of the study, ethical approval was obtained from the Institutional Review Board of the University of Cape Coast, Ghana. Also, a written informed consent was obtained from each female student after students were assured of anonymity and confidentiality and briefed on the study rationale. Anonymity and confidentiality were ensured by codifying students.

Issuance of Questionnaire

After random selection, each student was giving a structured questionnaire to be completed and returned on an appointed date.

Sample Collection

Randomly selected students were taught how to collect mid-stream urine samples and each student was then given two sterile, wide-mouthed and capped sample bottles to be returned on two appointed days, first with the completed questionnaire. Specifically students were advised to collect first urine (15 ml) in the morning of the appointed submission day. Urine samples upon receipt were immediately transferred into an ice container and conveyed to the laboratory for immediate processing and routine analysis. Urine samples were analyzed within 3 hours of collection to ensure integrity of samples, accurate identification of pathogens and also to avoid possible proliferation of pathogens and contaminants.

Urine Biochemical Analysis

The urine samples were analyzed semi-quantitatively to detect urine parameters including calcium oxalate, hematuria, proteinuria, leukocytes and nitrite, by dipstick method (Dirui industrial Company Limited China). Briefly, standard test strips, one/urine sample was dipped in each urine sample for 60 seconds, and then taken out. Developed color bands on strips were compared to standard color charts on the strip container and read according to manufacturer's instructions.

Microscopic Examination of Urine Samples

Dip slides of un-centrifuged urine samples were examined microscopically as previously described (Kildemoes et al., 2015; Labi et al., 2015).

Urine Culture

A loopful (0.002 ml) of well mixed urine was taken from each sample using a standard sterile wire loop and inoculated on a cysteine lactose electrolyte-deficient (CLED) agar (MAST, Germany). This was incubated aerobically at 35 to 37^{o}C overnight as previously described (Acquah et al., 2013; Boye et al., 2012). The bacteria strains from the selected plates were inoculated on blood agar and MacConkey agar followed by incubation for 18 to 24 h.

Bacteria Enumeration

By using spread plating technique, 0.1 ml of each of the inoculums (7th dilution) was spread on a plate count agar and the bacteria colonies counted using a Quebec colony counter (Reichert, USA), after 18 to 24 h of incubation. A single organism count ≥ 10^5 CFU/ml of two consecutively voided urine samples was considered significant (Nicolle et al., 2005) (that is, positive for ASB).

Bacteria Identification

On the basis of colony-specific morphology (that is, color, size and appearance) the different bacteria colonies that showed homogenous colony morphology were identified according to previously described methods (Gibreel et al., 2012). Subsequently, confirmatory identification of bacteria colonies was done by using Gram staining (Gibreel et al., 2012) and standard biochemical tests including citrate test, urease test, indole test, triple sugar iron (TSI) agar test, coagulase and catalase tests as previously described (Gibreel et al., 2012).

Antimicrobial Susceptibility Testing (AST)

The susceptibility of each bacteria isolate was determined by the modified Kirby-Bauer disc diffusion method (Bauer et al., 1966) with strict adherence to the Clinical and Laboratory Standards Institute guidelines. Briefly, ready-made antibiotic impregnated discs including Ampicillin (AMP) (10 µg), Cefuroxime (CRX) (30 µg), notrimoxazole (COT) (25 µg), tetracycline (TET) (30 µg), gentamycin (GEN) (10 µg), nalidixic acid (NAL) (30 µg), pipemidic acid (PPA) (30 µg), nitrofurantoin (NIT) (300 µg) (Axiom Laboratories, India) specific for urinary bacteria isolates were used. Plates were incubated aerobically at 37^{o}C for < 24 h after which various zones of inhibition were measured to the nearest millimeter (mm) and compared to reference standards to determine the susceptibility patterns of each bacterial isolate. As a quality control for the culture media used, the following American Type Culture Collection (ATTC) strains: *E. coli* ATTC 25922, *Staphylococcus aureus* ATTC 25923, and *Pseudomonas aeruginosa* ATTC 27853 (CLSI, Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing: 18th Informational Supplement, M100-S18. Wayne, PA: Clinical and Laboratory Standards Institute; 2008) were used. Acquired AMR for each isolate was estimated by following the interim recommendations of the International Expert Proposal for acquired resistance, defined as *in vitro* resistance to ≥ 1 antibiotic in ≥ 3 antimicrobial chemical classes (Magiorakos et al., 2012).

Data Analysis

Data were first entered into microsoft excel sheet and subsequently analyzed by using the Statistical Package for the Social Sciences (SPSS version 16.0). Data was summarized using descriptive statistics (proportions and percentages). Chi-square was used to determine associations between categorical variables where

Table 1. Age group-specific and overall ASB prevalence.

Age groups (years)	Number of students tested (%)^	ASB n (%)#; ASB*
16-18	2 (1.3)	0 (0.0) 0
19-21	104 (66.7)	12 (7.7) 11.5
22-24	36 (23.1)	2 (1.3) 5.6
≥ 25	14 (9.0)	1 (0.6) 7.1
Total	156 (100)	15 (9.6) 24.2

^Numbers in parenthesis represent number of students tested for ASB from each year group expressed as a percentage of the total number of students recruited (156); n = number of students who tested positive for ASB; # ASB positivity in each year group expressed as a percentage of the total number of students recruited (156) for the study; *ASB positivity as a percentage of the total number of students tested in each year group.

Table 2. Frequency of bacteria isolates and their point distribution.

Bacteria Isolates	Point Distribution of Isolates					
	ADH	ATL	KNH	VALCO	OGUAA	TOTAL
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
CoNS[a*]	2 (8.3)	2 (8.3)	2 (8.3)	2 (8.3)	1 (4.2)	9 (37.5)
K. pneumoniae[b]	2 (8.3)	0 (0.0)	1 (4.2)	2 (8.3)	1 (4.2)	6 (25.0)
S. aureus[a]	0 (0.0)	0 (0.0)	2 (8.3)	0 (0.0)	3 (12.5)	5 (20.8)
E. coli[b]	1 (4.2)	1 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.3)
S. marcescens[b]	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)	1 (4.2)	2 (8.3)
Total	5 (20.8)	3 (12.5)	6 (25.0)	4 (16.7)	6 (25.0)	24 (100.0)

[a]Gram - positive bacteria, [b]Gram - negative bacteria, [*]At least 1 isolate was detected from students of each of the 5 halls of residence, n = number of isolates. CoNS = Coagulase Negative Staphylococci*, E. coli* = *Escherichia coli*, *K. pneumoniae* = *K. pneumonia*, *S. marcescens* = *S. marcescens*, S*. aureus* = *S. aureus*. ADH = Adehye hall, ATL = Atlantic hall, KNH = Kwame Nkrumah hall.

applicable. $P \leq 0.05$ was considered statistically significant in all analysis.

RESULTS

Student Participation

Out of an initial 309 recruited female students, only 156 (~ 50.1%) fully complied (that is, returned both completed questionnaire and urine samples) and participated in the study, while the rest (~ 49.9%) either returned only completed questionnaire or failed to bring both completed questionnaire and urine sample.

ASB Positivity

Out of 156 female students drawn from five halls of residence, 15 (9.6%) tested positive (single colony count ≥ 10^5/organism) for ASB. Majority of the students (66.7%) were aged between 19 and 21 years, while only few (1.3%) were aged below 19 years (Table 1). Comparatively, ASB positivity was very high in the 19 to 21 age groups (Table 1). Out of the 156 cultured urine samples, a total of 24 bacteria isolates were identified comprising three Gram-negative and two Gram-positive bacteria species. Of the 24 isolates, CoNS (37.5%) was predominant, while *E. coli* and *S. marcescens* were the least (Table 2). At least one isolate of CoNS was isolated from urine sample of a student from each of the five halls of residence (Table 2). Microscopic detection of bacteria in un-centrifuge urine correlated with ASB culture positive samples. Five of the ASB culture positive samples had hematuria, while nitriuria was present in two of the ASB culture positive samples. None of the urine samples presented leukocyturia. Pus cells, calcium oxalate, yeast cells, and protein were the common urine inclusions detected (Table 3).

Antimicrobial Susceptibility Patterns

In all, eight antibiotics (Table 4) belonging to eight different chemical classes (aminoglycosides, cephalosporin, fluoroquinolones, nucleic acid inhibitors, penicillin, pyridopirimidines, sulfonamides, tetracycline) were used. *Serratia spp* and CoNS showed highest (75% in each case) pooled sensitivity to all the eight antibiotics tested; while *K. pneumonia* and *S. aureus* showed the highest (75 and 62.5%, respectively) pooled resistance (Table 4). All the isolates showed 100% sensitivity to nitrofurantoin and gentamycin. CoNS were 100% sensitive to all the antibiotics tested except ampicillin and cefuroxime (Table 4). *Klebsiella pneumonia* was 100% resistant to all the antibiotics tested except nitrofurantoin

Table 3. Urine inclusions detected from urine samples of students from the 5 halls of residence.

Urine Parameters	Hall of Residence					
	ADH	ATL	KNH	VALCO	OGUAA	TOTAL
	n = 36	n = 31	n = 25	n = 31	n = 33	N = 156
Calcium oxalate	7	3	1	1	0	12 (7.7)
Yeast cells	1	1	5	0	0	7 (4.5)
Bacteria[a]	2	0	0	0	2	4 (2.6)
Pus cells	5	7	7	4	9	32 (20.5)
RBC[b]	1	5	3	1	3	13 (8.3)
Nitrite[*]	1	0	2	1	0	4 (2.6)
Protein	0	2	3	0	2	7 (4.5)
Leukocytes[#]	0	0	0	0	0	

[a] Correlated with ASB culture positive (15) samples, [b] Five of the ASB culture positive (15) samples showed hematuria, [*] Only two of the ASB culture positive (15) samples tested positive for nitriuria, [#] None of the ASB culture positive (15) samples tested positive for leukocyturia. RBC = Red blood cells, WBC = White blood cells, ADH = Adehye hall, ATL = Atlantic hall, KNH = Kwame Nkrumah hall.

Table 4. Isolates and their susceptibility patterns to reference antibiotics.

Bacteria	Number of Isolates	Antibiotics									
Isolate		AMP[a]	TET	COT	NAL	NIT[b]	GEN[b]	CRX	PPA	Pooled % S	Pooled % R
[#]*E.coli*	2	2R	2S	2S	2R	2S	2S	2I	2R	50.0	37.5
[*]*Serratia spp.*	2	2R	2S	2S	2S	2S	2S	2R	2S	75.0	25.0
[#]*S. aureus*	5	5R	5S	5R	5R	5S	5S	5R	5R	37.5	62.5
[#]*K. pneumoniae*	6	6R	6R	6R	6R	6S	6S	6R	6R	25.0	75.0
[*]CoNS	9	9R	9S	9S	9S	9S	9S	9R	9S	75.0	25.0

[#] Isolate produced multiple drug resistance (MDR) (resistance to ≥ 3 antibiotics of ≥ 3 different chemical classes), [*] Isolates resistant to only 2 antibiotics belonging to 2 different chemical classes, [a] All isolates were resistant, [b] All isolates were sensitive. Pooled %S or %R was determined across rows as: number of S/8x100 or number of R/8x100. CoNS = Coagulase Negative Staphylococci*, E. coli* = *Escherichia coli*, *K. pneumoniae* = *Klebsiella pneumonia*, *S. marcescens* = *Serratia marcescens*, S*. aureus* = *Staphylococcus aureus*. AMP = Ampicillin, CRX = Cefuroxime, COT = Cotrimoxazole, GEN = Gentamicin, PPA = Pipemidic acid, NAL = Nalidixic acid, NIT = Nitrofurantoin, TET = Tetracycline. I = Intermediate, R = Resistance, and S = Sensitivity.

and gentamycin. *Staphylococcus aureus* was 100% resistant to all the antibiotics tested except tetracycline, nitrofurantoin and gentamycin (Table 4).

Behavioral Group-specific Risk Factors of ASB

Table 5 shows the elicited responses of students extracted from completed questionnaires. Most students whose urine samples tested positive for ASB were in their second year. Out of fourteen students who used vaginal douches, five of them were among those who tested positive for ASB. Out of fifty five students who said they use water closet by squatting on it, ten of them were among those who tested positive for ASB. Students mostly rated their water closets to be quiet clean. Most students claimed to cleanse their vagina from front to back, however, of the six students who said they cleanse their vagina after easing from back to front, two of them were among those students who tested positive for ASB. Most students responded that they washed their private parts with water after visiting the washroom. The panty fabric of most students was mostly made of cotton.

DISCUSSION

This study investigated ASB prevalence, common uropathogens associated with ASB and their antibiotic susceptibility patterns, as well as group-specific behavioral risk factors of ASB among resident tertiary female students. The study was occasioned by the apparent lack of data (ASB prevalence, antibiotic susceptibility patterns uropathogens, ASB risk factors) with specific reference to female tertiary students in Ghana compared to other most-at-risk groups such as children, pregnant women, menopausal women and diabetics, and this was confirmed by a thorough PubMed search. Essentially, ASB positivity was used as a direct measure of risk of asymptomatic and symptomatic urinary tract infections (Hooton, 2000; Ipe et al., 2016; Ipe et al., 2013). Out of 156 urine specimens tested (microscopy, dipstick, and urine culture) ASB positivity (prevalence) was 9.6%, with female students in the 19 to 21 year group recording the highest incidence. Comparatively, ASB prevalence of 9.6% is higher than those of other studies involving school girls including 1.6% (out of 500 adolescent school girls) (Emans et al.,

Table 5. Summary of questionnaire responses.

Variables	ADH	ATL	KNH	VALCO	OGUAA	TOTAL	*P*-value	X^2
	n = 36	n = 31	n = 25	n = 31	n = 33	N = 156		
Academic Level							0.019	24.27
Year one	26 (16.7)	20 (12.8)	24 (15.4)	25 (16.0)	26 (16.7)	121 (77.6)		
Year two	3 (1.9)	5 (3.2)	0 (0.0)	0 (0.0)	2 (1.3)	10[a] (6.4)		
Year three	4 (2.6)	5 (3.2)	1 (0.6)	0 (0.0)	2 (1.3)	12 (7.7)		
Year four	3 (1.9)	1 (0.6)	0 (0.0)	6 (3.8)	3 (1.9)	13 (8.3)		
Use of VD							0.791	1.697
Yes	5 (3.2)	2 (1.3)	2 (1.3)	3 (1.9)	2 (1.3)	14[b] (9.0)		
No	31 (19.9)	29 (18.6)	23 914.7)	28 (17.9)	31 (19.9)	142 (91.0)		
Pattern of WC use							0.820	4.391
Squat	10 (6.4)	11 (7.1)	8 (5.1)	15 (9.6)	11 (7.1)	55[*] (35.3)		
Sit	24 (15.4)	19 (12.2)	15 (9.6)	15 (9.6)	21 (13.5)	94 (60.3)		
Stand	2 (1.3)	1 (0.6)	2 (1.3)	1 (0.6)	1 (0.6)	7 (4.5)		
Nature of WC							0.005	22.2
Very clean	8 (5.1)	5 (3.2)	2 (1.3)	3 (1.9)	1 (0.6)	19 (12.2)		
Quite clean	24 (15.4)	26 (16.7)	17 (10.9)	19 (12.2)	30 (19.2)	116 (74.4)		
Dirty	4 (2.6)	0 (0.0)	6 (3.8)	9 (5.8)	2 (1.3)	21 (13.5)		
Cleansing style							0.423	12.28
F to B	32 (20.5)	27 (17.3)	20 (12.8)	26 (16.7)	30 (19.2)	135 (86.5)		
B to F	0 (0.0)	2 (1.3)	2 (1.3)	1 (0.6)	1 (0.6)	6[#] (3.8)		
Washing with H_20	4 (2.6)	1 (0.6)	2 (1.3)	4 (2.6)	0 (0.0)	11 (7.1)		
Anyhow	0 (0.0)	1 (0.6)	1 (0.6)	0 (0.0)	2 (1.3)	4 (2.6)		
Panty material							0.260	10.082
Cotton	33 (21.2)	29 (18.6)	21 (13.5)	31 (19.9)	32 (20.5)	146 (93.6)		
Nylon	0 (0.0)	1 (0.6)	1 (0.6)	0 (0.0)	1 (0.6)	3 (1.9)		
Both	3 (1.9)	1 (0.6)	3 (1.9)	0 (0.0)	0 (0.0)	7 (4.5)		

[a] Seven of the ASB positive (15) samples were from year two students, [b] Five of the ASB positive (15) samples were from students who use VD, [*] Ten of the ASB positive (15) samples were from students who squat on WC, [#] Only two of the ASB positive (15) samples were from students who cleanse from back to front. F: front, B: Back. VD: vaginal douch. ADH = Adehye hall, ATL = Atlantic hall, KNH = Kwame Nkrumah hall.

1979), 3.3% (out of 996 school girls aged between 14 and 17 years) (Oner et al., 2004), 5% (out of 796 specimens, of which majority were female university students) (Hooton et al., 2000), 5.4% (out of 314 female seventh graders) (Diamond et al., 1981) and even higher than the ASB prevalence range (1 to 5%) for healthy and pre-menopausal women (Nicolle, 2003). Given the relatively small sample size of the present study due to high drop-out rate (49.9%), it is suspected that the ASB prevalence in the study area may be higher than estimated. From this study, the clinically important uropathogens identified from urine samples in decreasing order were: coagulase negative *Staphylococci* > *Klebsiella pneumoniae* > *Staphylococcus aureus* > *Serratia marcescens* > *Escherichia coli* and this trend clearly shows that uropathogens associated with ASB are fast changing with recruitment of new uropathogens hitherto unknown.

The dominant isolate was *Coagulase Negative Staphylococci*, a typical member of which is *Staphylococcus saprophyticus*. *Staphylococcus saprophyticus* was implicated in ASB among sexually active females (Fihn et al., 1998), but it must be noted that while the present result regarding coagulase negative *Staphylococci* agrees with other reports (Tadesse et al., 2014; Vincent et al., 2013), it however stands at variance with several other studies (Al Sweih et al., 2005; Shakya et al., 2014; Sharma and Paul, 2012) which reported *E. coli* as the dominant bacteria strain implicated in ASB. It appears that ASB positivity distribution may be related to the endemicity of a specific uropathogen in a geographically-dependent manner as well as host-specific behaviors. These fundamental factors might in part explain the varied reports in relation to ASB etiology from diverse settings and different most-at-risk groups. The habit of uropathogens may alter urine physico-chemical properties, and this may be useful for diagnostic purposes, though sensitivity and specificity of such measures remain questionable. Proteinuria, nitriuria, and leukocyturia have been reported in ASB (Woodford et al., 2007), but as indicated earlier their accuracy for ASB diagnosis remains poor (Colgan et al., 2006; Kacmaz et al., 2006). For instance, Demilie and colleagues reported that leukocyturia does not always

correlate with ASB (Demilie et al., 2014), because other urogenital inflammatory conditions may also induce leukocyturia (Colgan et al., 2006; Kacmaz et al., 2006). Similarly, nitriuria may only be specific for ASB if the etiology of ASB is by a bacteriuric enterobacteria, beside this nitriuria is not specific for ASB, since many other uropathogens lack the enzymatic capacity to convert nitrates to nitrite (Kacmaz et al., 2006; Patel et al., 2005).

The present results showed proteinuria, nitriuria, and leukocyturia in some of the urine samples that tested positive for ASB as well as those that tested negative, and this observation does not only indicate poor predictive value of these urine parameters but also add credence to earlier reports (Colgan et al., 2006; Kacmaz et al., 2006). Prevention and treatment of infectious diseases is heavily threatened by AMR (Huttner et al., 2013). To help solve this major health threat, knowledge of endemicity of a particular bacteria strain and its AMR pattern is crucial for effective treatment and management of infectious diseases among most-at-risk groups. In Ghana, the Standard Treatment Guideline recommends use of ciprofloxacin for the treatment of urinary tract and blood-borne infections, while other antibiotics including ampicillin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole and third generation cephalosporins are widely used (Opintan et al., 2015). From this study both Gram-negative and Gram-positive bacteria isolates exhibited 100% sensitivity to gentamicin and nitrofurantoin, but 100% resistance to ampicillin, and this perhaps suggests that gentamicin and nitrofurantoin might well be suited for the treatment of ASBs of multiple etiology, while use of the penicillins must be reviewed.

The present observation agrees in part with an earlier finding, which reported that Gram-negative bacteria exhibited high sensitivity to gentamycin and nitrofurantoin (Jamie et al., 2002). Also, resistance to ampicillin by both Gram-positive and negative isolates corroborates an earlier report (Jamie et al., 2002) and this could be attributed to indiscriminate use of these older antibiotics in view of their cheaper prices (Opintan et al., 2015). Gentamicin (an aminoglycoside), is effective for the treatment of bacterial infections including those caused by Gram-negative bacteria (Tangy et al., 1985). Mechanistically, gentamicin inhibits bacterial protein synthesis; specifically it binds to the 30S ribosomal subunit to inhibit synthesis of essential proteins crucial for the survival and growth of the bacteria cells. On the other hand secondary metabolites of nitrofurantoin are active DNA degraders or nucleic acid inhibitors.

Following nitrofuran reductase-dependent reduction of nitrofurantoin in bacteria cells, a number of metabolites are released which cause damage to bacteria DNA to arrest growth and multiplication (Breeze and Obaseiki-Ebor, 1983; Sastry and Jayaraman, 1985). Interestingly, nitrofurantoin exerts selective effects on bacterial cells than those of mammals because bacterial cells rapidly activate nitrofurantoin (Breeze and Obaseiki-Ebor, 1983; Sastry and Jayaraman, 1985) than that of mammalian cells. Per the present results, gentamycin and nitrofurantoin were 100% effective against all the isolates, perhaps suggestive of the need to reconsider the chemical classes of these two antibiotics for novel combined therapies. In short, host drug-taking behavior plays crucial role in the development of AMR just as host-specific behavior influence transmission of infectious diseases. Many factors have been linked to the relatively higher predisposition of females to ASB than their male counterparts. It was indicated that the relatively short and straight anatomy of the female urethra to the vagina makes it more exposed to trauma during sexual intercourse and could lead to retrograde ascent of bacteria up the urethra into the bladder (Boye et al., 2012; Feitosa et al., 2009), while others have associated female preponderance to ASB to unprotected sex, age and genitourinary disorders as well as use of estrogen-based contraceptives (Hooton et al., 1996). However, from the present study it was observed that host behavior, specifically improper use of WCs such as squatting on the WC seats coupled with poor sanitary conditions of washrooms may have in part accounted for ASB positivity among females at the study area. Although a direct association between ASB positivity and risk factors was not vigorously pursued statistically, however, matching of questionnaire responses of students (Table 5) with their ASB positivity status revealed that some of the students who tested positive for ASB engaged in some improper hygienic practices. For instance, some of the ASB positive students squat on WCs instead sitting on it; cleanse their vagina from back to front instead of from front to back, as well as use of vaginal douches, which possibly could have disadvantaged them in terms of ASB risk compared to their colleagues who observed proper personal hygiene.

This study was limited by our inability to identify the specific strains of the isolates, especially CoNS, in view of its dominance in this study as well as the recent emergence of *Staphylococcus saprophyticus*, a major pathogenic strain of CoNS in symptomatic urinary tract infections (Lo et al., 2015). It is important that future study establish whether *S. saprophyticus* and other pathogenic strains are major part of CoNS isolates from the study area. Also, high drop-out rate was a major constraint to the study as many students were unwilling to partake for reasons that could be personal, religious or cultural. Future studies should use novel ways to enroll large sample size and also characterize the specific strains of the isolates. Put together, an overall ASB prevalence of 9.6% out of 156 female students indicates that ASB is quiet high among the resident female students at the study area.

CONCLUSION

ASB positivity was estimated to be 9.6% from 24 bacteria

isolates with CoNS as predominant isolate. All the bacteria isolates were susceptible to nitrofurantoin and gentamicin but resistant to ampicillin. Regular health education on the risk of infectious diseases and screening should be incorporated into the healthcare programs of tertiary institutions in Ghana.

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