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Extended Spectrum Beta Lactamase Producing Gram Negative Bacteria Contaminating Medical Examination Equipment and Clinical Coats at Bugando Medical Centre, Tanzania: Implication for Infection Prevention and Control

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Abstract

Background: Infections due to extended spectrum beta lactamase (ESBL) producing gram negative bacteria (GNB) have been a global challenge. The magnitude of ESBL producing GNB contaminating medical examination equipments (MEEs) and clinical coats was not clearly known.

Objective: This study determined the magnitude and associated factors of ESBL producing GNB contaminating MEEs and clinical coats at Bugando Medical Centre, Mwanza.

Methods: This was a cross sectional hospital based study involving 407 swabs specimen from clinical coats (n=157) and MEE (n=250), conducted from April to July 2017. Swabs were cultured on plain MacConkey agar and MacConkey agar supplemented with 2µg/ml cefotaxime. Isolated GNB were identified by in-house biochemical identification tests. ESBL production was confirmed by double disc synergy technique.

Results: Overall, the magnitude of GNB contaminating clinical coats and MEE was 35.9% (146/407), and out of 146 GNB, 34 (23.3%) were ESBL producers. Clinical coats were highly contaminated with GNB and ESBL producing bacteria than MEE; 57.3% (90/157) vs. 22.4% (56/250), $p<0.001$ and 24.7% (23/93) vs. 18.9% (11/58), $p<0.001$, respectively. ESBL producers were highly resistant to gentamicin 73.5% (25/34) and highly sensitive to meropenem 97.1% (33/34).

Conclusions: The magnitude of MEEs and clinical coats contamination with ESBL producing GNB is high. Clinical coats are significantly more contaminated. Therefore, MEE and clinical coats should also be potential niches of focus in the infection prevention and control strategies in this hospital.

Keywords: clinical coats, ESBL, medical examination equipment, Tanzania

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Introduction

Approximately half of Gram negative bacterial infections at Bugando Medical Centre (BMC) in Mwanza, Tanzania are due to extended spectrum beta lactamase (ESBL) producers[1, 2]. ESBL producing Gram negative bacteria (GNB) causing infections can be transmitted from one patient to another via many different routes, the most common being; healthcare workers' contaminated hands, clinical coats and medical examination equipments (MEEs) in routine use [3-6]. Clinical coats and MEE become contaminated with ESBL producing GNB when they come into contact with infected or colonized patients' surfaces [7, 8]. Lack of disinfection and/or ineffective disinfection of MEE after being used in a patient can acts as a potential transmissions route of these pathogens from infected or colonized patient to uninfected patients [6]. Healthcare workers' hands can become contaminated with GNB and even ESBL producers when they are in direct contact with infected patients, MEE and clinical coat surfaces; and subsequently act as reservoir or vehicle of transmission to patients [5, 9, 10].

It has been documented that ESBL producing GNB can contaminate MEEs and medical personnel's clinical coats [5, 7]. Further reports indicated that, clinical coats if carried outside of healthcare facility premises can play a role of transmitting nosocomial pathogens to the community settings [5]. One study reported that, 19.1% physicians' clinical coats were contaminated with multidrug resistant GNB [10]. Another study reported 67% of MEEs were contaminated with multidrug resistant GNB; of which, *Acinetobacter baumanii* was predominantly isolated [11]. Emphasis on proper handling and disinfection of the clinical coats and MEEs would therefore minimize cross-contamination and cross-transmission of nosocomial pathogens, and thereby improves patients' safety [5].

Despite high prevalence of ESBL attributable infections, increased morbidity and mortality at BMC [1, 2, 12]; there is limited information on ESBL contaminating clinical coats and MEE. This study determined the magnitude and associated factors of ESBL producing GNB contaminating MEEs and clinical coats in the context of guiding comprehensive infection prevention and control strategies.

Materials and methods

This was a cross sectional study conducted from April to July 2017, involving 407 specimens; 157 swabs form healthcare workers' and medical students' clinical coats and 250 swabs from medical equipment (thermometers, stethoscopes and blood pressure (BP) machines' cuffs) carried by 113 health care workers. This study was conducted at Bugando Medical Center; tertiary referral, teaching and consultant hospital with bed capacity of about 1000 beds. The study included MEEs in use and worn clinical coats by medical personnels. This study was given research and ethical clearance approval by the CUHAS/BMC Research & Ethical Committee (CREC 273/2017 and 278/2017).

Sample collection

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Medical examination equipment (stethoscopes' diaphragms, BP machine cuffs and thermometers) and clinical coats (upper parts of pockets) were swabbed by using wet sterile swabs and then inoculated into Stuart transport media (Amies transport medium, Thermo Scientific, U.K). Swabs specimens were transported to the CUHAS Multipurpose Laboratory within two hours of collections for microbiological processing.

Culture and bacteria identification

Swab specimens were cultured onto MacConkey agar plates (HiMedia, India) and in-house 2µg/ml cefotaxime supplemented MacConkey agar (MCA-C) plates (HiMedia, India), and then incubated aerobically in 37°C for 18 to 24 hours. MCA-C was purposely used for screening of ESBL production among GNB. Bacterial identification to species level was done considering growth morphology on culture plates and biochemical characteristics by using in-house prepared biochemical identification tests; fermentation of sugars and production of CO_2 and H_2S gases in Triple Sugar Iron; Suphur indole motility; Urease production; Citrate utilization and Oxidase tests [13]. Control organisms used were; *E. coli* ATCC 25922 (none ESBL producers) and *E. coli* ATCC 35218 (ESBL producer).

Confirmation of ESBL production

ESBL confirmation was done by using double discs synergy whereby ceftriaxone 30µg and ceftazidime 30µg were seeded edge to edge with amoxycillin/clavulanic acid 30µg about 15mm distance [14, 15]. Enhanced zones of inhibitions of ceftazidime and/or ceftriaxone discs towards amoxycillin/clavulanic acid were considered as confirmed ESBL phenotype [15].

Antibiotic susceptibility testing (AST)

AST was done for ESBL producing bacteria only using disc diffusion technique on Muller Hintoin agar, MHA (HiMedia, India). Suspension of ESBL producing isolates equivalent to 0.5% McFarland turbidity was swabbed onto MHA, gentamicin 10µg, ciprofloxacin 30µg, amikacin 30µg and meropenem 10µg discs (HiMedia, India) were seeded. Zones of inhibitions were recorded and interpreted as recommended by CLSI [16].

Results

Social demographic information of study participants

A total of 407 specimens; 250 MEEs swabs were collected from 113 healthcare workers and medical students; and 157 clinical coats swabs were collected from 157 healthcare workers and medical students. Median age (inter-quartile range) years of MEEs participants was 25(24-33) years. Approximately 55.8% (63/113) of study participants were female. Distribution of participants in MEEs study were; 60% (68/113) medical students, 26.8% (30/113) nurses and 13.2% (15/113) doctors. Median age (inter-quartile range) years of participants whose clinical coats were swabbed was 27(25-39) years; with nearly two third being males, 64.5% (101/157). Distribution of participants whose clinical coats were swabbed was 44.6% (70/157), 38.9%

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(61/157) and 16.6% (26/157) for medical students, doctors and intern doctors, respectively (Table I).

Culture results

Overall prevalence of GNB contaminating clinical coats and MEE was 35.9% (146/407). Clinical coats were found to be more contaminated than MEE; 57.3% (90/157) vs. 22.4% (56/250), p <0.001. From clinical coats, three cultures had dual pathogens; two *Citrobacter freundii* and one *Klebsiella pneumonia* making a total of 93 isolated GNB (Figure 1). And from MEE, two cultures had dual pathogens; one *Escherichia coli* and one *Klebsiella pneumoniae* making a total of 58 isolated GNB. This makes a total of 151 GNB contaminating MEE and clinical coats. *Pseudomonas aeruginosa* was found to be predominant GNB contaminating both clinical coats and MEE; 23.7% (22/93) and 31.0% (18/58) respectively (Figure 2).

ESBL producing GNB

A total of 22.5% (34/151) ESBL producing bacteria were found to contaminate clinical coats and MEE. ESBL producing bacteria was found to contaminate more clinical coats than MEE; 24.7% (23/93) vs. 18.9% (11/58), p = 0.001. *Citrobacter freundii*, 43.5% (10/23) was found to be predominant ESBL producing GNB contaminating clinical coats while *Acinetobacter* spp., 36.4% (4/11) was predominant ESBL producers contaminating MEE (Figures 1 and 2).

Percentage sensitivity of ESBL producing GNB

The overall sensitivity of ESBL producing GNB was; 52.7% (18/34), 26.5% (9/34), 40% (14/34) and 97.1% (33/34) for amikacin, gentamicin, ciprofloxacin and meropenem respectively. *Acinetobacter* spp isolated from MEEs was the only ESBL producing GNB resistant to meropenem.

Association factors for ESBL contaminating MEEs and clinical coats

Univariate regression analysis show that ESBL contaminating MEEs statistically to be associated with type of medical examination equipment whereby, BP machine cuffs were significantly more contaminated, 63.6% (7/11), p = 0.017 (Table II).

Discussion

This study found that, GNB are highly contaminating clinical coats and MEEs; with clinical coats being more contaminated. Healthcare workers' gloved hands are in direct contact with pathogens when attending patients, especially patients with wound infections, therefore may potentially become contaminated. Whenever hand hygiene is not stringent, contaminated hands can contaminate clinical coats especially in the pockets' entries[17]. This may explain why clinical coats were highly contaminated than MEEs. This emphasize on adherence to the basic hand hygiene measures among healthcare workers and medical students to prevent the possibility of cross-transmission of these deadly MDR bacteria [18].

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Pseudomonas aeruginosa was the most predominant GNB isolated from both clinical coats and MEEs followed by *Enterobacter aerogenes* and *Acinetobacter* spp for clinical coats only and *Citrobacter freundii* and *Acinetobacter* spp for MEEs only. Similar results have been reported previously [19-21]. These pathogens are known to be implicated in healthcare associated infections [22-25], and they can survive in inanimate and dry surfaces for weeks to months [26-28]. Predominance of *Pseudomonas aeruginosa* has also been documented in various reports to be the commonest colonist and/or pathogen at BMC [29, 30].

The current study found that, ESBL producing GNB were highly contaminating clinical coats and MEEs, clinical coats being highly contaminated. As previous observed [31], ESBL producing *Pseudomonas aeruginosa* and *Acinetobacter* spp are frequently isolated from clinical coats; while ESBL producing *Citrobacter freundii* and *Enterobacter aerogenes* were frequently isolated form MEEs.

In both cases; GNB and ESBL producing GNB contaminating MEEs and clinical coats, *Pseudomonas aeruginosa* was prevalently isolated. This might be due to the ability of *Pseudomonas aeruginosa* in biofilm formation, and developed intrinsic resistant mechanism to normal concentrations and short term exposure to disinfectants in frequently use in healthcare settings [32, 33].

The preponderance of medical students' examination equipments being significantly associated with contamination by ESBL producing GNB; may be linked to their frequently use and in some cases being infrequently disinfected as individuals in this group are still undergoing training [34, 35]. This in turn emphasizes on the importance of monitoring them closely on adherence to hand hygiene and aseptic techniques during their training. Moving with clinical coats outside hospital premises, storing clinical coats at home, and washing without antiseptic detergents increases the risk of ESBL producing GNB contaminating clinical coats. Moreover, moving with contaminated clinical coats outside of hospital premises increases the risk of transmitting ESBL producing GNB to the community as reported previously [8]. Antiseptic detergents help killing microbes in clothes and clinical coats washed without antiseptic detergents are more likely to carry ESBL producing GNB [36].

In the current study, ESBL producing GNB were highly sensitive to meropenem, moderately sensitive to amikacin and less sensitive to gentamicin and ciprofloxacin. Similarly, less sensitivity of ESBL producing GNB to gentamicin and ciprofloxacin were previously reported [37, 38]. Being cheap (ciprofloxacin) and first line of treatment (gentamicin), make these antibiotics to be highly misused and therefore more likely to develop resistance [39]. As observed previously, ESBL producers are highly sensitive to meropenem [1, 2, 40]. Extremely high cost and infrequently availability of meropenem in most of healthcare settings and pharmacies in least developed countries, limit its misuse and extreme use, therefore bacteria exhibit very low or no resistant against meropenem. However some bacterial species have been reported to resist meropenem [40], as currently seen in this study, one *Acinetobacter* spp was resistant to meropenem. *Acinetobacter* spp has been reported to simultaneously equip itself with

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intrinsic and extrinsic mechanisms of antibiotics resistant. This includes, diminished production of outer membrane porin therefore increasing its outer membrane impermeability, possession of efflux pump and production of Amp-C beta lactamase [41, 42].

Contaminated clinical coats and MEEs can act as vehicles to carry and transmit ESBL producing GNB from one patient to another, to oneself (medical personnel) and to the community to become the ultimate source of community acquired multidrug resistant bacteria as reported before [5, 6, 8, 10].

Conclusion and recommendation

The current study found high prevalence of ESBL producing GNB contaminating MEEs and clinical coats; of note clinical coats were highly contaminated than MEEs. ESBL producing GNB contamination of MEEs was associated with type of medical equipment.

Therefore, MEE and clinical coats should also be potential niches of focus in the infection prevention and control strategies in this hospital. Moreover, we recommend further molecular studies to characterize ESBL producing GNB contaminating MEEs and clinical coats and their possible link with healthcare acquired infections (HCAIs) in this hospital.

Authors' contributions

VS, MFM, JS and SEM conceived and designed this study. DJK and PBS did data collection. VS, DJK, PBS, MFM and JS did laboratory procedures. VS and MFM analyzed study data. All authors critically reviewed the first draft of this manuscript. All authors approved the final draft of the manuscript.

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Conflicts of interest

All authors declare no conflict of interest.

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Table I; Basic information of study participants whose clinical coats and MEEs were evaluated for ESBL producing GNB

MEEs participants' information			
Variable		**Frequency (n/N)**	**Percent (%)**
Median age (IQR) years		25(24-33)	-
Sex	Male	50/113	44.2
	Female	63/113	55.8
Occupation	Medical students	68/113	60
	Nurses	30/113	26.8
	Doctors	15/113	13.2
MEEs type	Stethoscope	112/250	44.8
	Thermometer	71/250	28.4
	BP machine cuff	67/250	26.8
Awareness and behavior	Awareness of MEEs disinfection	104/113	91.6
	MEEs disinfection after attending patient	73/113	64.8
	Using of 70% as disinfectant	69/113	61.2
Clinical coats participants' information			

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Variable		Frequency (n/N)	Percent (%)
Median age (IQR) years		27(25-39)	-
Sex	Male	101/157	64.3
	Female	56/157	35.7
Occupation	Medical students	70/157	44.6
	Doctors	61/157	38.9
	Intern doctors	26/157	16.6
Moving coats out of hospital premises	No	60/157	38.2
	Yes	97/157	61.8
Coat sharing	No	127/157	80.9
	Yes	30/157	19.1
Storage of coats after working hours	Hospital	51/157	32.5
	Home	89/157	56.7
	Hostel	17/157	10.8
Antiseptic washing of coats	No	109/157	69.4
	Yes	48/157	30.6

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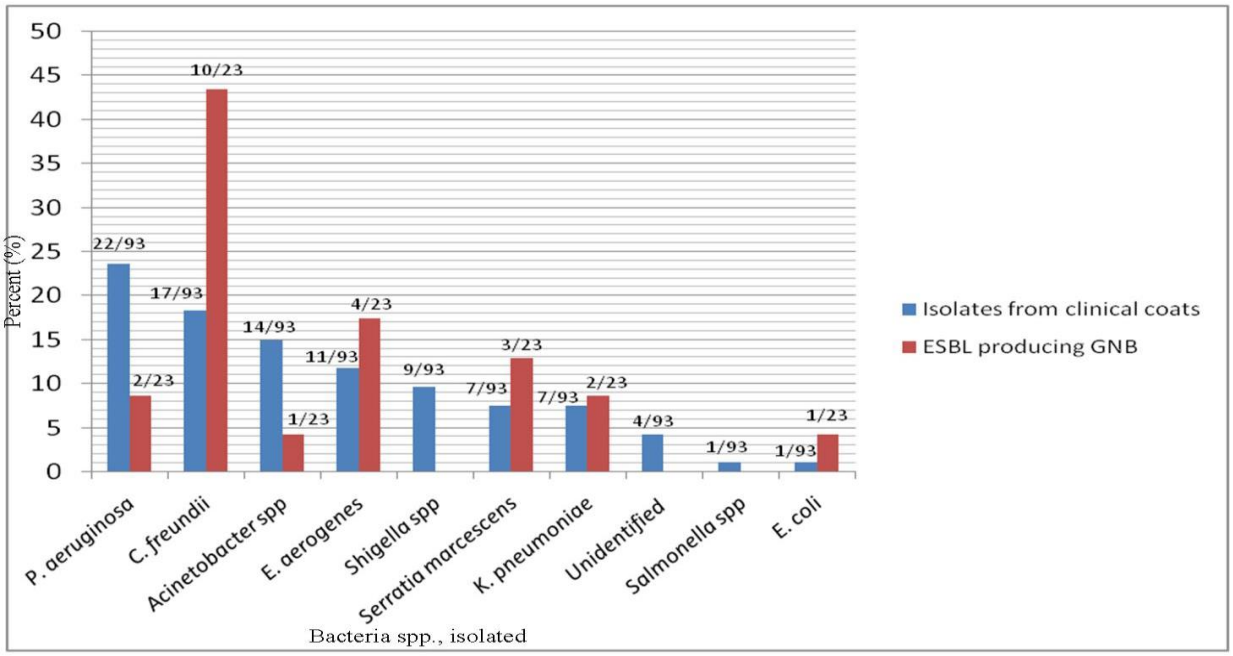

Figure 1; Distribution of bacterial spp., and ESBL producing GNB isolated clinical coats

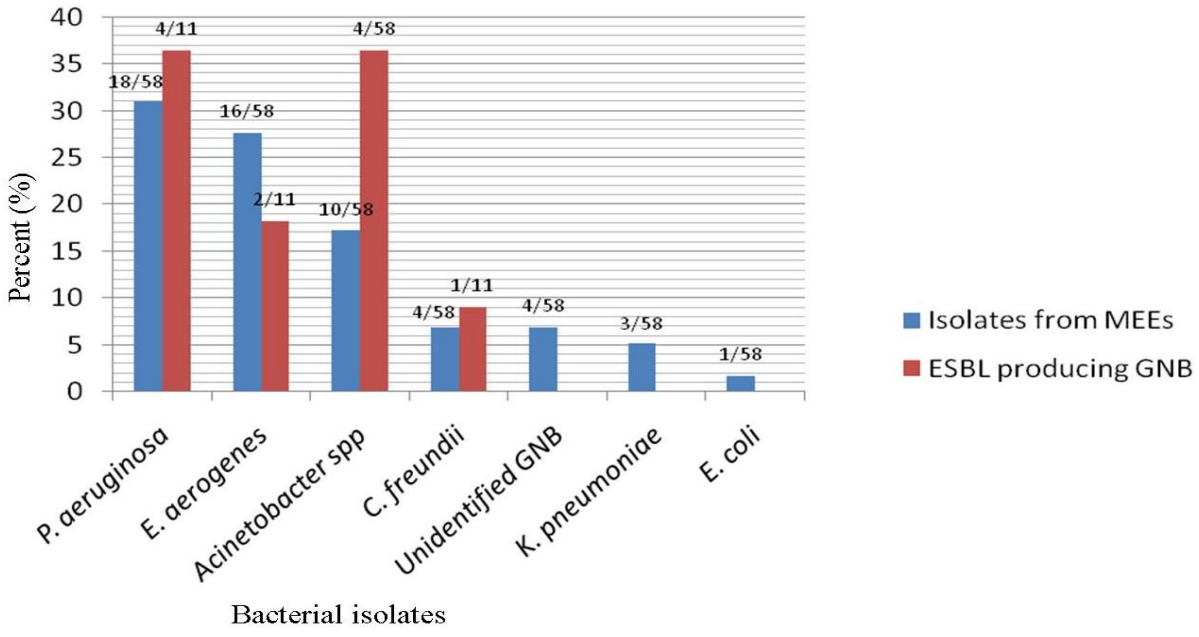

Figure 2; Distribution of bacterial spp., and ESBL producing GNB isolated from medical examination equipments

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Table II; Association of ESBL producing GNB contaminating clinical coats and MEEs with variables

MEEs contamination		**Univariate regression analysis**	
Variables		**n/N (%)**	**P value**
Professional levels	Medical students	7/11(63.6%)	0.212
	Nurses	1/11(9.09%)	
	Doctors	3/11(27.3%)	
Disinfection awareness	No	0/11 (0%)	
	Yes	11/11(100%)	0.304
Frequency of use of MEE	Frequently	9/11(81.8%)	0.756
	Weekly	1/11(9.09%)	
	Monthly	1/11(9.09%)	
Frequently disinfection of MEE	Yes	3/11(27.3%)	
	No	8/11(72.7%)	0.573
Disinfectant agent	70% alcohol	6/11(54.6%)	0.870
	None	5/11(45.5%)	
Type of equipment	Thermometer	1/11(9.09%)	
	Stethoscope	3/11(27.3%)	
	BP machines' cuff	7/11(63.6%)	**0.017**
Clinical coats contamination			
Variables		**n/N (%)**	**P value**

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Professional level	Medical student	8/23(36.4%)	
	Intern doctors	5/23(22.7%)	
	Doctors	9/23(40.9%)	0.624
Outside hospital areas	Yes	14/23(60.9%)	0.827
	No	8/23(34.8%)	
Sharing coats	Yes	2/23(8.7%)	
	No	20/23(86.9%)	0.584
Storage	Hospital	8/23(36.4%)	
	Home	14/23(63.6%)	0.209
Antiseptic wash	Yes	4/23(18.2%)	
	No	18/23(81.8%)	0.168