RESEARCH ARTICLE

Use of 16s rRNA to identify non-lactose-fermenting bacilli and molecular detection of ESBL resistance genes associated with hospital-acquired infection in Soba University Hospital, Sudan

[version 1; peer review: 1 approved]

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Abstract

Background: Non-lactose-fermenting gram-negative bacilli (NLFGNB) have become significant nosocomial pathogens and often exhibit intrinsic multidrug resistance. Sequencing of 16s rRNA genes could be utilized for robust identification of NLFGNB. This study aimed to identify resistant NLFGNB associated with hospital-acquired infections using 16s rRNA sequencing and to detect the extended-spectrum β -lactamase (ESBL) genes of isolates in Soba Hospital, Khartoum State, Sudan.

Methods: A prospective, cross-sectional, laboratory-based study was conducted from October 2017 to March 2018 at the Microbiology Department of Soba University Hospital. A total of 100 randomly selected NLFGNB samples were isolated from blood and urine during the time of the study. All the isolates were identified using standard biochemical tests and antimicrobial sensitivity testing, 16s rRNA gene sequencing, and bioinformatics techniques.

Results: The biochemical tests revealed that, out of the 100 NLFGNB isolates, the *Pseudomonas* species was predominant (57 isolates), followed by gram-negative bacilli (33 isolates), *Coccobacilli* (9 isolates) and *Coliform* (1 isolate) species. Sequencing of 16s rRNA genes identified all the resistant isolates at the species level: *Pseudomonas aeruginosa* (26%), *Acinetobacter baumannii* (22%), *Burkholderia cepacia* (13%), *Stenotrophomonas maltophilia* (10%), *Enterococcus* species (*E. faecalis*, *E. faecium*) (10%), and other GNB (*Acinetobacter variabilis*, *Klebsiella pneumoniae*, *Morganella morganii*, *Escherichia fergusonii*, *Enterobacter hormaechei* and *Pseudomonas stutzeri*) (19%). The antimicrobial susceptibility tests indicated that 31 isolates were resistant to at least three classes of antibiotics and contain the highest level of ESBL resistance genes.

Conclusions: *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

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were the most widely recognized NLFGNB identified from hospital-acquired infections in Soba hospital. Among the NLFGNB, antimicrobial resistance and ESBL resistance genes were observed at a high frequency.

Keywords

Gram-negative bacilli, non-lactose fermenting, 16S rRNA, ESBL, hospital-acquired infection, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, nosocomial infection.

This article is included in the **Pathogens** gateway.

This article is included in the **Antimicrobial Resistance** collection.

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Introduction

Hospital-acquired infections, also known as nosocomial infections (NIs), by gram-negative bacteria are a major threat to health worldwide (Karaïskos & Giamarellou, 2014). These bacteria are consistently evolving and developing novel mechanisms of resistance to commonly used antibiotics, especially when these antibiotics are overused, which generates a selection pressure (Peleg & Hooper, 2010). Increasingly, non-lactose-fermenting gram-negative bacilli (NLFGNB) have become significant pathogens in healthcare facilities (Akbar *et al.*, 2014; Hiltunen *et al.*, 2017). The public health risk of NLFGNB is growing rapidly worldwide; consequently, warnings of the disturbing spread of anti-infection-resistant microorganisms causing NIs, which have cost lives and resources, have also risen (Djordjevic *et al.*, 2017; Leski *et al.*, 2016; Ranjbar *et al.*, 2018).

The over-prescription and use of antimicrobial agents may be playing a major role in the escalating number of NIs (Mataseje *et al.*, 2016; Prasert *et al.*, 2020; Rabirad *et al.*, 2014). Bacteria are identified phenotypically and characterized by biochemical tests; however, these tests are not as accurate as required (Bush & Jacoby, 2010). Advanced techniques in molecular biology and sequencing approaches are needed for the discovery, identification, and characterization of emerging and re-emerging pathogens (Wilson *et al.*, 2014). 16s rRNA gene sequencing offers a practical and reliable molecular identification method (Brown-Elliott *et al.*, 2006; Claridge, 2004). 16s rRNA gene sequence analysis can better identify poorly described, recognize inadequately depicted, infrequently isolated, or phenotypically deviant strains, can be routinely utilized for identification of bacteria, and can prompt the acknowledgment of novel pathogens and non-cultured bacteria (Case *et al.*, 2007; Crosby & Criddle, 2003; Dahllöf *et al.*, 2000; Reller *et al.*, 2007). A significant function for 16s rRNA gene sequencing is to identify precisely assembled living organisms for additional study (Goldenberger *et al.*, 1997; Jalava *et al.*, 1995). Gram-negative organisms are involved in both network- and emergency clinic-procured contaminations (Mataseje *et al.*, 2016; Prasert *et al.*, 2020). NLFGNB infections cause a significant public health problem in hospitals, especially when they develop multi-drug resistance (Kaye & Pogue, 2015; Lochan *et al.*, 2017). These microorganisms can develop several mechanisms of resistance, including β -lactamase creation, overexpression of multi-drug efflux pumps, target site mutations, and reducing the permeability of the external membrane (Djordjevic *et al.*, 2017).

The correct identification of these bacteria by conventional microbiology methods is often limited by phenotypic misidentification (AbdulWahab *et al.*, 2015; Alby *et al.*, 2013; Plongla *et al.*, 2016). The spread of extended-spectrum β -lactamase (ESBL)-making strains restricted the range of antimicrobial agents that could be used to treat patients effectively and raised concerns for control of infections caused by NFGNB and limitation of treatment to prohibitively expensive antibiotics (Manyahi *et al.*, 2017; Ranjbar *et al.*, 2018; Zhang *et al.*, 2009). The mutation of TEM-1, TEM-2, and SHV-1 β -lactamases produced the ESBLs, which were discovered over 1980–1990 and first detected in Western Europe (Bubpamala *et al.*, 2018;

Bush *et al.*, 1995). DNA sequencing technologies offered a sensitive diagnostic tool that improved the detection, identification, and characterization of drug-resistant bacteria (Livermore, 1995; Ranjbar *et al.*, 2018).

NLFGNB is commonly associated with hospital-acquired infections; therefore, identification of the species and the study of antimicrobial susceptibility patterns are imperative. ESBL genes are the most common resistance genes associated with gram-negative bacilli. Thus, it is essential to determine both the prevalence of ESBL among NLFGNB and the recognizable proof of microscopic organisms at the species level, which is essential to determine the most effective treatment and best guide case management (Bush *et al.*, 1995; Livermore, 1995).

In Sudan, recognizable proof of NLFGNB at the species level is difficult to achieve by conventional methods. Alternative strategies, (e.g., API or VITEK) are not affordable; therefore, the current study was undertaken to provide more sensitive and accessible methods that require 16s rRNA to detect and confirm the bacterial pathogens separated from patient samples in Soba University Hospital in Khartoum, Sudan.

Methods

Bacteria isolation, identification, and antimicrobial sensitivity testing

A prospective, cross-sectional, laboratory-based study was conducted from September 2017 to February 2018. The study conducted in the Microbiology Department of Soba University Hospital and the Institute of Endemic Diseases, University of Khartoum, Sudan. A total of 100 clinical isolates from specimens of urine and blood were collected from hospitalized patients (Pourhoseingholi *et al.*, 2013). All isolates that are NLFGNB were included and isolates that are gram positive and/or lactose fermenter excluded in this study. Blood isolates totaled 64 (64%) and urine totaled 36 (36%). All 100 strains of NLFGNB sub-cultured on blood, chocolate, and MacConkey agar and incubated at 37°C overnight for re-identification, at which point they were assessed using standard microbiological strategies (such as morphology, microscopy, and biochemical tests set out by Nagy *et al.*, (2018)). Quality control strains were utilized in biochemical tests and antimicrobial susceptibility testing of *E. coli* (ATCC #25922) and *P. aeruginosa* (ATCC #27853). NFLGB colonies and gram-negative bacteria were selected. Antibiotic susceptibility testing was accomplished by way of Kirby-Bauer disk-diffusion; all isolates were swabbed on Muller-Hinton agar, placed on antibiotic disks, and incubated at 37°C for 18–24 hours. All isolates were tested against the following antibiotics: ceftriaxone (CRO; 30 μ g), ceftazidime (CAZ; 30 μ g), cefuroxime (CXM; 30 μ g), cefotaxime (CXM; 30 μ g), cotrimoxazole (COT; 25 μ g), amikacin (AK; 10 μ g), ciprofloxacin (CIP; 5 μ g), Cefepime combined with amoxycillin/clavulanate (AMC; 30 μ g), and imipenem (IPM; 10 μ g). Results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines (Eigner *et al.*, 2005; Moubareck *et al.*, 2009).

Molecular identification

DNA was extracted from cultured specimens using guanidine chloride and underwent PCR using a thermal cycler (Analytik

Jena Biometra TADVANCED, Germany), by using specific primers, such as the universal primer 16s rRNA, and specific primers for ESBL genes, such as CTX-M, SHV, and TEM (Table 1). PCR reaction was completed in a total reaction volume of 25 µL (5 µL Master mix of Maxime RT PreMix kit [iNtRON Biotechnology, Seongnam, Korea], 0.6 µL forward primer, 0.6 µL reverse primer, 2 µL DNA, and 16.8 µL deionized sterile water). The following cycle conditions were used: initial denaturation step at 94°C for 5 min, after which there were 30 cycles of denaturation at 94°C for 45 seconds, primer toughening temperature (as indicated in Table 1) for 45 seconds, and extension at 72°C for 60 seconds, with a final elongation step at 72°C for 5 min. Products were electrophoresed in 2% agarose gel in 1X TBE containing 2.5 µL of ethidium bromide (20 mg/mL) at 100 V for 40 min. The amplified product was distinguished by contrasting with a 100-base-pair standard DNA ladder (iNtRON BIOTECHNOLOGY, Seongnam, Korea) and the bands were visualized under UV (analytikjena® Biometra BDACompact, Germany). Purified the PCR results of 16SrRNA and Sanger sequencing was performed by Macrogen Company (Seoul, Korea). Then nucleotide sequences of the genes 16SrRNA accomplished were scanned for similarity sequence using nucleotide BLAST for species identification (Petti, 2007).

Bioinformatics analysis

Nucleotide sequences of the 16s rRNA gene were assessed for sequence similarity and species identification using NCBI Nucleotide BLAST. MEGA X software was used for conducting statistical analysis of molecular evolution and for constructing phylogenetic trees of bacteria identified in the study.

Statistical analysis

Data were analyzed using SPSS version 20.0 and Microsoft Excel. Cross tabulation was used to present the relation data, quantitative data were performed to discover the contrasts between bacterial isolates with resistance to at least one class of antibiotics by samples (blood and urine).

Ethical approval

The study protocol was approved by the ethics committee of the Institute of Endemic Diseases, University of Khartoum, and the permission was obtained from the managers of Soba

University Hospital, under reference number IEND_REC 12/2017. Participation in this study was entirely voluntary and all participants read and signed informed consent forms; where children were assessed, their parents provided written informed consent.

Results

Demographic distribution

The largest proportion of specimens were collected from the NICU ward, comprising 42 blood and 4 urine specimens (91% and 8.7%, respectively). In total, ten blood and nine urine specimens were then collected from the pediatric ward (52.6% and 47%, respectively), followed by specimens from the renal unit (six blood and five urine samples), the medicine ward (one blood and nine urine samples), the ICU unit (five blood and three urine samples), and the surgery ward, which held the lowest number of specimens (six urine and no blood samples). The number of samples collected from inpatients from different wards are shown in Table 2. Demographic details, alongside identification of bacterial isolates and resistance genes, are available as *Underlying data* (Al Hag, 2020).

Identification of bacterial isolates

A total of 60 out of 100 isolates (60%) were recognized by traditional and biochemical strategies; the remaining 40% could not be identified. The isolate *Pseudomonas* spp. (54%) was most common, followed by other GNB (40%) then *Coccolibacilli* (6%). Molecular identification using 16s rRNA sequencing showed that the most common *Acinetobacter* spp. that was isolated was *Acinetobacter baumannii* (25.3%), while the most common oxidase positive isolate was *Pseudomonas aeruginosa* (34.8%). *Pseudomonas* spp. (12.6%), *Burkholderia cepacia* (6.8%), *Stenotrophomonas maltophilia* (4.8%), and other gram-negative bacilli, including *Morganella morganii* and *Enterobacter hormaechei*, account for 10%. The isolates of *Enterococcus* spp. account for (3.3% of *E. faecium* and *E. faecalis*), *Escherichia fergusonii* and *Klebsiella pneumoniae* (2.4%) were excluded from the results (Figure 1).

Antimicrobial resistance pattern

NLFGNB isolates with antimicrobial resistance pattern are shown in Figure 2. Isolates tested using the disk diffusion method showed the highest percentage of resistance to be 98% and

Table 1. Primers used for PCR bacteria identification.

Primers	Sequences (5' to 3')	*MT/°C	Length (bp)	References
Universal 16s rRNA	F-5'AGAGTTTGATCCTGGCTCAG3' R-5'CTA GGC TACCTTGTT GAC GA3'	58°C	1500	Srinivas & Bhadru (2015)
bla TEM	F-5' TCG GGG AAA TGT GCG CG 3' R-5' TGC TTA ATC AGT GAG GCA CC 3'	57°C	971	Hoşoğlu et al. (2007); Saladin et al. (2002)
bla SHV	F-5'GGTTATGCGTTATATTCGCC-3' R-5' TTAGCGTTGCCAGTGCTC-3'	57°C	797	Weill et al. (2004)
bla CTX-M	F-5'SCSATGTGCAGYACCAGTAA-3' R-5'CCGCRATATGRTTGGTGGTG-3'	57°C	550	Eckert et al. (2004)

Table 2. Number of samples collected from inpatients according to hospital ward.

Hospital Ward	Sample Type	
	Blood N (%)	Urine N (%)
NICU*	42 (91.3%)	4 (8.7%)
Medicine	1 (10.0%)	9 (90.0%)
Pediatrics	10 (52.6%)	9 (47.4%)
Renal unit	6 (4.5%)	5 (45.5%)
Surgery	0 (0.0%)	6 (100.0%)
ICU	5 (2.5%)	3 (37.5%)
Total	64 (64.0%)	36 (36.0%)

Figure 1. Distribution of non-lactose-fermenting gram-negative bacilli isolated from hospitalized patients in Soba Hospital. Other gram-negative bacilli include *Morganella morganii* and *Enterobacter hormaechei*.

94.5% against ampicillin and cefotaxime, respectively, followed by cephalexin (90.7%), amoxicillin/clavulanic acid (90.1%), ceftriaxone (88.4%), and ceftazidime (84%). Co-trimoxazole and nitrofurantoin resistance were recorded to be present in 74.3% and 75.1% of isolates, respectively. Resistance rates to ciprofloxacin was 50.1%, gentamicin was 52.5%, and amikacin was 22.3%. Imipenem and meropenem were the most efficient antibiotics tried, with resistance rates of 17.2% and 20.2%, respectively. The examination of the antimicrobial susceptibility pattern of these isolates demonstrated that NLFGNB showed high rates of multidrug resistance, being resistant to most third-generation cephalosporins, as observed in *P. aeruginosa* and *A. baumannii* (99% and 98%, respectively) (Testing, 2017).

Detection of ESBL resistance genes

PCR amplifications were performed to recognize three virulence genes using their appropriate primers. The most frequent gene found in species is SHV (~51% of total isolates), followed by CTX-M (~43%) and TEM (~20%). All bacterial species that were isolated had one or two ESBL genes; therefore, they show a high level of antibiotic resistance (Table 3) (Rhoads et al., 2012).

BLAST for resistance bacteria

The alignment of DNA sequences for NLFGNB isolates identified them at the species level and contrasted with public databases resulted in the retrieval of two sequences of various species which displayed identical similarity scores; thus, the

Figure 2. Frequency of antibiotic resistance in bacterial isolates (n = 100). Names of antimicrobial agent were abbreviated as follows: imipenem (IPM), meropenem (MER), ceftazidime (CAZ), ceftriaxone (CTR), cefotaxime (CXM), cephalixin (CN), ciprofloxacin (CIP), gentamicin (GEN), cotrimoxazole (COT), nitrofurantoin (NIT), amoxicillin/clavulanic acid (AMC), ampicillin (AMP), and amikacin (AK).

Table 3. Frequency of extended-spectrum β -lactamase results among different strain isolates.

Bacterial Species Isolates	CTXM, N (%)		SHV, N (%)		TEM N (%)	
	Positive	Negative	Positive	Negative	Positive	Negative
<i>Pseudomonas spp</i>	26 (41%)	37 (59%)	31 (49%)	32 (51%)	13 (21%)	50 (79%)
<i>Coccobacilli</i>	4 (40%)	6 (60%)	6 (60%)	4 (40%)	3 (30%)	7 (70%)
<i>Other Gram-negative bacilli</i>	13 (50%)	13 (50%)	13 (50%)	13 (50%)	4 (15%)	22 (85%)
Total	43 (43%)	57 (57%)	51 (51%)	49 (49%)	20 (20%)	80 (80%)

isolate was not assigned to a single taxon but was reported as belonging to either of the two species. The sequence of an isolate showed 99.99% identity to sequences of *Pseudomonas stutzeri* and *Pseudomonas spp.*, whereas *B. cepacia* represented 99.86%, *S. maltophilia* represented 99.88%, *E. fergusonii* represented 99.72%, and other gram-negative bacteria represented 95.88% identity.

Discussion

NLFGNB were previously considered to be contaminants but have now emerged as significant healthcare-associated pathogens (Malini *et al.*, 2009). *Pseudomonas* and *Acinetobacter* species are known to be the regular nosocomial pathogens (Gales *et al.*, 2001; Tortoli *et al.*, 2001). In this study, the most common NLFGNB isolated was *P. aeruginosa*, followed by *A. baumannii*, which is similar to the results obtained by

Sarkar *et al.* (2018) and Malini *et al.* (2009), who reported *P. aeruginosa* as the most common isolate, followed by *A. baumannii* (Malini *et al.*, 2009; Wauters & Vanechoutte, 2015). Other NLFGNB, such as *B. cepacia*, *S. maltophilia*, and *Stenotrophomonas pavanii* vary from study to study, both in terms of their genera and prevalence. However, their role as opportunistic pathogens in immunocompromised and debilitated individuals has been invariably established (Chawla *et al.*, 2013). Strains isolated by biochemical methods were recorded a *Pseudomonas spp.*, *Coccobacilli*, and other GNB. The sequencing test identified the isolates to many strains, such as *P. aeruginosa*, *A. baumannii*, *B. cepacia*, *S. maltophilia*, and other GNB. The prevalence of ESBLs in this study showed SHV to be the highest level of the total sample, followed by CTX-M, with TEM being the lowest of the resistance isolates. ESBLs are enzymes that

demonstrated resistance to the third generation of cephalosporin. As these enzymes are plasmid-encoded, the spread of bacterial resistance disseminates rapidly (Jena *et al.*, 2017). ESBLs are responsible for resistance against β -lactam antibiotics, such as penicillin, cephalosporin, monobactams, and sometimes also carbapenems (Cantón *et al.*, 2008). Resistance of NLFGNB and multidrug resistance have risen widely according to numerous studies. *B. cepacia* is another NLFGNB that colonizes and infects patients with chronic bacteremia and UTI. It is known to cause infections in hospitalized patients and, once infected, is hard to eliminate (Akbar *et al.*, 2014; Chawla *et al.*, 2013; Plongla *et al.*, 2016).

In this study, the isolate of *B. cepacia* recorded 6.8% of the non-fermenters isolated from blood and urine culture. *B. cepacia* is known for its inherent resistance to numerous beta-lactam drugs, including aminoglycosides, colistin and polymyxin B, first-line therapeutics against serious pseudomonas infections (Rahbar *et al.*, 2010). This isolate showed maximum (100%) susceptibility to imipenem, in accordance with Sidhu *et al.* who also reported 100% susceptibility to imipenem. Similarly, in a study done by Grewal *et al.*, (2017) and Gautam *et al.*, (2009). *B. cepacia* isolates showed excellent susceptibility to imipenem and Meropenem.

Three strains of *S. maltophilia* were isolated during the study; it is now considered a common non-fermenter that causes infection in hospital settings (Verweij *et al.*, 1998). Our study has shown the isolation of this bacterium in 6.4% of isolates. Earlier A'Court and Garrard reported *S. maltophilia* to account for 5% of nosocomial pneumonias (A'Court & Garrard, 1992). It showed 100% susceptibility to some of the antibiotics, notably ciprofloxacin. Similar results were obtained by Malini *et al.* (2009).

The majority of our isolates were resistant to ampicillin (98%), followed by cefotaxime (94.5%) then amoxicillin/clavulanate acid (90.1%). Resistance rates also rise in ciprofloxacin (50.1%), gentamicin (52.5%), and amikacin (22.3%). Imipenem and meropenem were the most effective antibiotics tested (Malini *et al.*, 2009; Rahbar *et al.*, 2010), with 100% sensitivity to ampicillin, cefotaxime, and ciprofloxacin.

A phylogenetic tree for the most common NLFGNB shows the relationships among various species, with their phylogeny based on similarities and differences in their genetic characteristics.

This study covered this gap by using advanced molecular techniques to identify, characterize, and determine the spread of hospital-associated bacterial infections to permit infection control personnel to recognize potential sources of pathogens more effectively, and encourage physicians, healthcare

workers, and stakeholders to develop treatment and control strategies against these rapidly evolving organisms.

Conclusion

In conclusion, NLFGNB in communities and hospitals can help inform antibiotic treatment. This study showed a significantly higher prevalence of *P. aeruginosa* and *A. baumannii* among NLFGNB associated with hospital infections. The isolation of MDR (*P. aeruginosa*) and MDR (*A. baumannii*) in the present study raises the concern of rapidly emerging antibiotic resistance in this group of bacteria in Sudan. Proper screening of non-fermenters in nosocomial settings and regular assessment of their antibiotic susceptibility profiles are suggested for effective management of infections and limitation of the emergence of multidrug resistance. Assessment of 16S rRNA gene sequences permits bacterial identification that is stronger, more reproducible and more exact than that obtained by phenotypic testing. Thus, when ordinary methods do not yield an excellent (or very good) species identification, non-fermenter GNB should be exposed to 16S rRNA gene sequencing if adequate species assignment is of concern. Since the spread of MDR and ESBL-producing NLFGNB isolates reduces treatment options and increases hospital cost, it is important to keep up to date on prevailing resistant patterns of any locality, which will help determine a suitable antimicrobial therapy.

Data availability

Underlying data

The 16S rRNA sequences generated in this study are available from GenBank under sequential accession numbers MW034199–MW034227.

Figshare: Data information 1_original.xlsx. <https://doi.org/10.6084/m9.figshare.13077398.v1> (Al Hag, 2020).

This project contains the following underlying data:

- Data information 1_original.xlsx. (Demographic information with details on bacterial strain and resistance profile.)
- Data information 2_original.xlsx. (Results of PCR genotyping for each sample.)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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The current study is a cross-sectional, laboratory-based study. Approved methods such as biochemical tests and antimicrobial sensitivity testing, 16s rRNA gene sequencing, and bioinformatics techniques were all used in the study. The current study has used variable methods and analysis tools like; conventional methods, antimicrobial sensitivity testing, molecular technique, and Bioinformatics software. The statistical analysis and its interpretation were appropriate. However, the small sample size of 100 isolates may hinder some limitations on the current study. Possible sensitivity analysis on data for molecular identification, and bioinformatics analysis may have improved the uptake of the current study findings. The use of outcome measure as quantitative data was performed to discover the contrasts between bacterial isolates with resistance to at least one class of antibiotics by samples (blood and urine). The source data underlying the results were available to ensure full reproducibility of the study. For instance, the 16S rRNA sequences generated in this study were available from GenBank under sequential accession numbers MW034199– MW034227.

The conclusions of this current original research were consistent with data reported, and the paper has cited current literature, for instance relevant papers published in 2020. The current research has identified resistant Non-Lactose-Fermenting Gram-Negative Bacilli (NLFGNB) (100 sample) associated with hospital-acquired infections using 16s rRNA sequencing (57% *Pseudomonas species*). The current work has detected the extended-spectrum β lactamase (ESBL) genes of isolates. Resistant was evident in 31 isolates (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*). This study showed a significantly higher prevalence of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* among NLFGNB associated with hospital infections. The isolation of MDR *Pseudomonas aeruginosa* and MDR *Acinetobacter baumannii* in the present study raises the concern of rapidly emerging antibiotic resistance in this group of bacteria particularly in Sudan. The resistance of NLFGNB represents a major challenge for hospital acquired bacterial infection with possibility of multi-drug resistant. Therefore, attempts to identify the genetic pattern and expression of the associated genes in the above-mentioned resistance can have implications on

the detection of the relevant genes of extended-spectrum β -lactamase (ESBL).

The above-mentioned findings may assist in improving our understanding of the resistant pattern of NLFGNB which is associated with many hospital-acquired infections. The current study showed important clinical relevant information that is relevant to teaching and could be of value to critical thinking learning. The study findings may contribute to future clinical research specially in academic centres with distinct investigations on bacterial resistant.

Finally, the uptake of the current study needs further research work to generalize the research findings that could be emulated by similar clinical trials.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Professor of Clinical medicine and expert clinical pharmacist. Specialized in all clinical research and peer review of scientific content and merits of publications.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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