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ORIGINAL ARTICLE

Co-Inheritance of Aminoglycoside Acetyltransferases and Extended-Spectrum β-Lactamases among Gram-Negative Clinical Isolates in Khartoum, Sudan (2023)

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ABSTRACT

Background: The global spread of β-lactamase-producing Gram-negative rods (GNRs) poses a significant threat to the treatment of bacterial infections, as these pathogens are resistant to most β-lactam antibiotics. Aminoglycosides have emerged as an alternative treatment option. This study aimed to detect aminoglycoside acetyltransferase genes *AAC (3)-II* and *AAC (6')-Ib* among extended-spectrum β-lactamase (ESBL)-producing GNRs isolated from clinical specimens in Sudan.

Methods: A cross-sectional study was conducted on 143 Gram-negative clinical isolates collected from hospitals in Khartoum State between August and September 2022. The isolates were screened for ESBL production using the double-disc synergy test. DNA was extracted using a modified boiling protocol, and multiplex PCR was performed to detect the *AAC (3)-II* and *AAC (6')-Ib* genes.

Results: Among the 143 isolates, 100 were confirmed as ESBL-producing GNRs. The predominant ESBL-producing organisms included *Pseudomonas aeruginosa*, *Acinetobacter spp*., *Escherichia coli*, Proteus spp., Klebsiella spp., and Salmonella spp. The AAC (6')-Ib gene was detected in 23% (23/100) of the isolates, whereas the *AAC (3)-II* gene was not detected in any isolates (0/100).

Conclusion: A high prevalence of the *AAC (6')-Ib* gene was observed among ESBL-producing Gram-negative isolates in Sudan, indicating an increased resistance pattern that requires urgent attention to mitigate its clinical and public health impact.

Key words: β- lactamase, Gram negative, antimicrobial resistance, Sudanese

INTRODUCTION

The rise of antibiotic resistance, particularly among gram-negative bacteria, threatens global health. The emergence of resistance to newer β-lactams, fluoroquinolones, and aminoglycosides has compromised treatment efficacy [1]. Aminoglycoside resistance is primarily mediated by transferable modifying enzymes, rRNA methylases, and efflux systems [2]. Aminoglycoside-modifying enzymes, including aminoglycoside acetyltransferases (*AACs*), nucleotidyl transferases (*ANTs*), and phosphoryl transferases (*APHs*), inactivate aminoglycosides [3]. *AACs*, specifically the *AAC* (3) and *AAC (6')* subfamilies, are prevalent in gram-negative bacteria and exhibit substrate specificity [3].

All over the globe, β-lactam antibiotics, comprising carbapenems, monobactams,

cephalosporins, and penicillin's, have become commonplace [4]. Resistance to β-lactams is multifactorial, primarily mediated by beta-lactamases that cleave the β-lactam ring [5]. Bacteria employ three main strategies to evade β-lactam effects: altered penicillin-binding proteins, efflux pumps, and beta-lactamase production [6].

This study aims to investigate the co-occurrence of aminoglycoside acetyltransferase genes *AAC (3)-II* and *AAC(6')-Ib)* among extended-spectrum β-lactamase (ESBL)-producing gram-negative rods (GNRs) isolated from clinical specimens in Khartoum, Sudan.

METHODS

Study Design and Sampling

This descriptive cross-sectional laboratory-based study included 143 Gram-negative clinical isolates from various specimens (wounds, urine, blood, CSF, and body aspirates) collected from Khartoum state hospitals between August and September 2022.

Ethical Approval

The study was approved from al-Neelain university ethical committee, and federal ministry of health, Sudan.

Bacterial Identification

Isolates were re-identified morphologically on blood agar and MacConkey agar. Gram staining and biochemical tests (Kligler iron agar, motility, citrate utilization, urease production, Indole production, methyl red, Voges-Proskauer, and oxidase production) confirmed the identification.

ESBL Screening

Double disc synergy testing detected ESBL-producing strains. Amoxicillin-clavulanic acid, ceftazidime, and cefotaxime discs were placed 30 mm apart. ESBL production was indicated by enhanced inhibition zones after 48-hour incubation at 37°C [7].

DNA Extraction

Genomic DNA extraction uses a modified boiling protocol [8]. Briefly, 1000 µL cell suspension (~10^7 cells/mL) was boiled at 100°C for 5 minutes, and then centrifuged. The supernatant was mixed with cold absolute ethanol, centrifuged, and the pellet was washed with 70% ethanol. The nucleic acid pellet was re-suspended in TE buffer.

Detection of Resistance Genes

PCR conditions: 5 µL extracted DNA, 1 µL primers, 14 µL distilled water, and PCR Master Mix (Maxime PCR Premix Kit). Thermocycling: 94°C (5 min), 30 cycles (94°C, 30 s; 56°C, 30 s; 72°C, 1 min), and 72°C (5 min) [11].

Gel Electrophoresis

PCR products were separated on 2% agarose gel, stained with ethidium bromide, and visualized under UV light.

Statistical analysis: data was analyzed using SPSS

RESULTS

A total of 143 Gram-negative clinical isolates were included in this study. These isolates were re-identified and screened for extended-spectrum beta-lactamase (ESBL) production using the double-disc synergy test. Among them, 100 isolates demonstrated ESBL production. Urine samples exhibited the highest frequency of ESBL-producing Gram-negative rods (31 isolates), followed by wound swabs (23 isolates), body aspirates (19 isolates), cerebrospinal fluid (CSF) samples (16 isolates), and blood cultures (11 isolates) (Figure 1).

The 100 ESBL-producing Gram-negative rods were further identified, revealing the predominant ESBL-producing organisms as Pseudomonas aeruginosa, Acinetobacter spp., Escherichia coli, Proteus spp., Klebsiella spp., and Salmonella spp., respectively (Figure 2).

All clinical isolates were tested for the presence of aminoglycoside acetyltransferase genes AAC (3)-II and AAC (6')-Ib. Among these, 23% (23/100) of the isolates were positive for the AAC (6')-Ib gene, while the AAC (3)-II gene was not detected (0/100) (Table 1). ESBL-producing Gram-negative rods isolated from wound swabs showed the highest frequency of the AAC (6')-Ib gene, followed by isolates from urine, CSF, and body aspirates (Table 2).

Table 3 details the distribution of the AAC (6')-Ib gene among different Gram-negative organisms. Pseudomonas aeruginosa was the most frequent producer of the AAC (6')-Ib gene (8/23), followed by Escherichia coli (6/23), Acinetobacter spp. (5/23), Klebsiella

spp. (3/23), and Proteus spp. (1/23). The AAC (6')-Ib gene was not detected among Salmonella spp. (0/23)

Table 1: Specific primers for detection of AAC (3)-II and AAC (6')-Ib genes (Multiplex PCR):

Primer	Sequence (5'→3')	Size (bp)	Reference
AAC (3)-II (F)	ATATCGCGATGCATACGCGG	877	[9]
AAC (3)-II (R)	GACGGCCTCTAACCGGAAGG		
AAC (6')-Ib (F)	TTGCGATGCTCTATGAGTGGCTA	472	
AAC (6')-Ib (R)	CTCGAATGCCTGGCGTGTTT		

Table 2: Prevalence of AAC (6')-Ib Gene Among Clinical Specimens

Specimen	AAC (6')-Ib Positive	Percentage
Wound swabs	9/23	39.1%
Urine	7/23	30.4%
CSF	4/23	17.4%
Body aspirates	3/23	13%
Blood culture	0/23	0%

Table 3: Prevalence of AAC (6')-Ib Gene bacterial spp

Bacteria	AAC (6')-Ib Positive	Percentage
Pseudomonas aeruginosa	8/23	34.8%
Escherichia coli	6/23	26%
Acinetobacter spp	5/23	21.7%
Klebsiella spp	3/23	13%
Proteus spp	1/23	4.3%
Salmonella spp	0/23	0

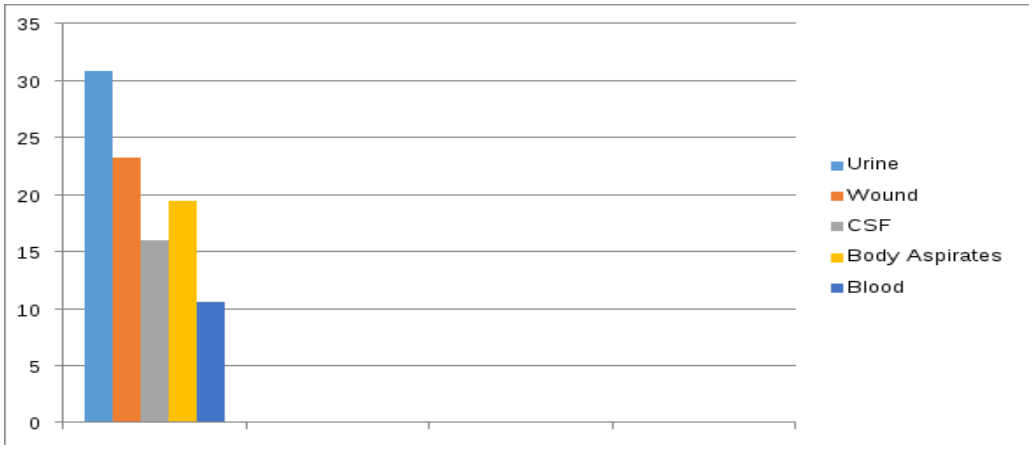

Figure (1): Distribution of ESBL-producing GNRs among clinical specimens

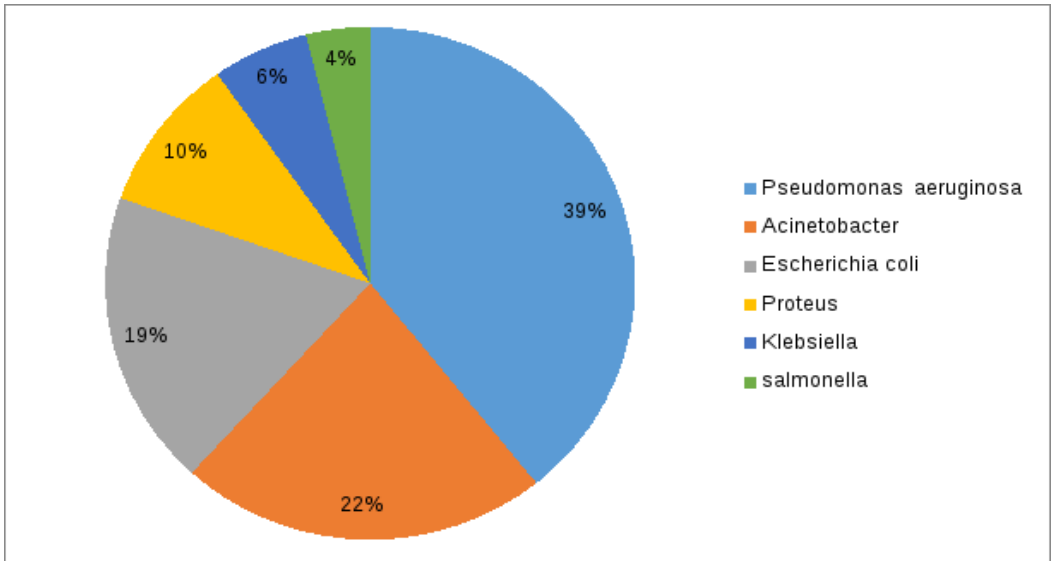

Figure (2): Prevalence of ESBL production among GNRs clinical isolates**.**

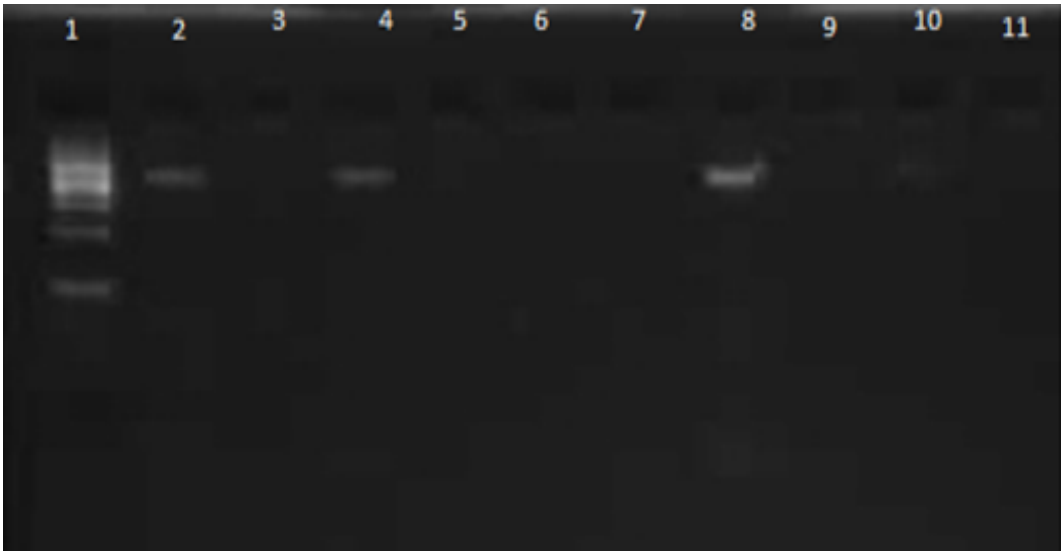

Figure (3): Post Gel electrophoresis figure for AAC (6) lb lane (1) is a ladder, lane (2, 4, 8) are positive isolates, and lane (3, 5, 6, 7, 9, 10, 11) are negative isolates.

DISCUSSION

Our study revealed that 23% of β-lactamase-producing Gram-negative rods (GNRs) carried the *AAC (6')-Ib* gene, underscoring its significant prevalence in our region. This gene, known to mediate aminoglycoside resistance through acetylation, is increasingly reported worldwide [16]. Interestingly, none of the isolates tested positive for the *AAC (3)-II* gene, suggesting a distinct regional profile of aminoglycoside resistance mechanisms. The high prevalence of *AAC (6')-Ib* in our study aligns with reports from other countries, such as India (43.5%) [12] and Saudi Arabia (56.5%) [11], indicating that this resistance mechanism is widespread in certain geographic regions. Notably, this is the first documented report of aminoglycoside acetyltransferases among extended-spectrum β-lactamase (ESBL)-producing GNRs in Sudan, marking a critical contribution to local antimicrobial resistance epidemiology [15].

Furthermore, our data revealed that Pseudomonas aeruginosa was the most frequent ESBL producer in our cohort (39%). This finding contrasts with previous reports where Klebsiella pneumoniae and Escherichia coli were predominantly implicated [10]. This shift in pathogen prevalence could reflect changes in local antibiotic usage patterns, infection control practices, or environmental factors. It emphasizes the importance of continuous surveillance to monitor evolving resistance trends and pathogen distributions.

Our findings are consistent with global studies highlighting the increasing prevalence of the *AAC (6')-Ib* gene. For instance, a 2014 study from Switzerland reported prevalences of 22.8% for *AAC (3)-IId* and 11.8% for *AAC (6')-Ib*-cr among E. coli isolates [13]. Similar studies in Sudan have reported high ESBL production rates in *Acinetobacter baumannii* (89%) and *Klebsiella pneumoniae* (78%), as noted by Dirar et al. [10]. These results underscore the growing burden of resistance and the necessity of regional surveillance programs to guide therapeutic strategies.

The absence of the *AAC (3)-II* gene in our isolates may reflect specific bacterial strain distributions in Sudan or limitations in the genetic profiling methods employed. Comparatively, the global spread of *AAC (6')-Ib* highlights its increasing clinical significance, demanding novel strategies to address this resistance mechanism [14]. The contrast between our findings and studies identifying Klebsiella pneumoniae and E. coli as dominant ESBL producers may indicate regional variations in healthcare-associated infections or antibiotic prescribing practices.

The high prevalence of *AAC (6')-Ib* raises critical concerns for managing infections caused by ESBL-producing GNRs. Aminoglycosides, often used in combination with other antibiotics, may lose efficacy in treating such infections. Our findings underscore the urgent need for regular surveillance and antimicrobial stewardship programs tailored to local resistance patterns [17].

From a therapeutic standpoint, alternative strategies such as novel β-lactam/β-lactamase inhibitor combinations or targeted therapies guided by susceptibility testing should be considered. Additionally, molecular diagnostic tools should be incorporated into routine clinical workflows to enable rapid detection of resistance genes and optimize treatment decisions. This approach could reduce reliance on empiric regimens and minimize the spread of multidrug-resistant organisms [16].

While this study provides valuable insights, several limitations should be acknowledged. First, the analysis was restricted to a single center, which may not fully capture the broader epidemiological trends in Sudan. Second, the absence of the *AAC (3)-II* gene might reflect limitations in the bacterial strains tested or the molecular techniques employed. Third, the study's cross-sectional design does not allow for tracking temporal trends in resistance mechanisms.

Addressing these limitations in future studies will provide a more comprehensive understanding of antimicrobial resistance in Sudan and its determinants.

Future research should expand the scope of surveillance to include larger and more diverse samples from multiple healthcare centers across Sudan. Investigating the genetic diversity of *AAC* genes and other

resistance determinants in different bacterial species would provide a deeper understanding of resistance mechanisms. Longitudinal studies tracking the emergence and spread of resistance genes over time are also essential to inform evidence-based policies. Collaboration between microbiology laboratories, hospitals, and public health authorities will be critical in tackling the growing challenge of antimicrobial resistance.

Conclusion

This study highlights a significant prevalence of the *AAC (6')-Ib* gene among β-lactamase-producing GNRs in Sudan, reflecting the growing threat of aminoglycoside resistance in the region. These findings emphasize the need for ongoing surveillance, targeted antimicrobial stewardship programs, and the integration of molecular diagnostics into routine practice. By improving our understanding of local resistance patterns, clinicians can optimize treatment regimens, ultimately enhancing patient outcomes and combating the global challenge of antimicrobial resistance.

Recommendations

To address the growing challenge of antimicrobial resistance, the following recommendations are provided:

1. Regular Surveillance: Establish continuous surveillance programs for ESBL-producing Gram-negative rods (GNRs) and aminoglycoside resistance genes in healthcare facilities across Sudan including the demographic data as well as risk factors.
2. Antibiotic Stewardship Programs: Develop and implement antibiotic stewardship programs focusing on the rational use of antibiotics, proper dosing, and stringent prescription practices.
3. Strengthening Infection Control: Enhance infection control measures, including adherence to hand hygiene protocols, sterilization practices, and isolation procedures for infected patients.
4. Advanced Molecular Studi**es**: Conduct further research utilizing molecular techniques to characterize ESBL-producing GNRs and identify resistance genes, providing insights for targeted interventions.
5. Interdisciplinary Collaboration**:** Encourage collaboration among healthcare professionals, including clinicians, microbiologists, and epidemiologists, to create and execute effective strategies against antimicrobial resistance.
6. Public Awareness Campaigns: Initiate awareness programs targeting healthcare workers, patients, and the public to highlight the dangers of antimicrobial resistance and promote judicious antibiotic use.

These recommendations aim to support healthcare policymakers, researchers, and practitioners in mitigating the escalating problem of antimicrobial resistance and improving patient outcomes.

Conflict of interest: None.

Financial Disclosures: None.

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