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Prevalence of *Acinetobacter spp.* in Intensive Care Units of Selective Hospitals at Khartoum State, Sudan

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Abstract Multidrug resistant (MDR) *Acinetobacter spp.* has emerged as an important cause of nosocomial infections with increased morbidity and mortality, evidently frequent in intensive care unit (ICU). The unique environment of ICU, artificial ventilation and other invasive procedures, exposure to antibiotics, colonization pressure, and underlying illness facilitate the spread of this species in ICU. Microbiological culture and antibiotic susceptibility testing was considered as one of the proper methods to assess the magnitude of this problem This study aimed to evaluate the prevalence and antimicrobial resistance profile of *Acinetobacter spp.* in selective hospital's intensive care units of Khartoum state, Sudan. A total of 980 different types of samples were processed by routine microbiological investigation and Antimicrobial susceptibility testing of the *Acinetobacter* isolates was performed by the disk diffusion method as recommended by Clinical Laboratory and Standards Institute CLSI. Samples were then confirmed as *Acinetobacter spp.* by using PCR. As a result, consecutive non duplicate 77 *Acinetobacter sp.* were isolated out of a total 340 pathogenic Gram negative isolates (22.6 % prevalence). Cephalosporins, aminoglycoside, fluoroquinolones and carbapenems are becoming completely ineffective while colistin was the only effective choice.

Keywords: *Acinetobacter spp., ICU, Antimicrobial resistance, PCR*

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1. Introduction

Acinetobacter is a gram-negative, strictly aerobic, non-fermenting coccobacillus belonging to the Moraxellaceae family [1]. It was first described in 1911 as Micrococcus calcoaceticus. Since then, it has had several names, becoming known as Acinetobacter in the 1950s [2]. Species belonging to its genus are opportunistic pathogens with increasing relevance in both community-acquired and nosocomial infections, particularly among patients in intensive care units (ICUs) and high-dependency units (HDUs) [3,4,5,6]. These organisms have been implicated in various infections, including ventilator-associated pneumonia, endocarditis, meningitis, and infections of the skin, soft tissues, urinary tract, and those originating from prosthetic devices [7]. Its natural habitats are water and soil, and it has been isolated from foods, arthropods, and the environment [8,9]. Although *Acinetobacter* has emerged as an important pathogen, little is known about its natural reservoirs and habitat. Pathogenic members of the genus Acinetobacter contribute to the normal flora of human skin, upper respiratory tract, and gastrointestinal tract. The clinical consequences of *Acinetobacter* infections range from minimal to moderate to severe. *A. baumannii*, along with two other genetically closely related species (genomic species 3 and 13TU), is almost exclusively associated with human infection and is phenotypically difficult to differentiate routinely in clinical laboratories. Hence, the group is known as *A. baumannii A. calcoaceticus*-complex (Abc complex), and is often regarded *A. baumannii* in clinical practice as [10,11]. Although many consider *A. baumannii* to be ubiquitous, not everyone agrees. It is considered to be commensal with humans, and colonization is well documented. Therefore, the switch from colonization to infection is more favorable than it would be from more distant environmental sources [12].

Acinetobacter spp. has capacity to exchange genetic material which facilitates to acquire antimicrobial resistance determinants among the species [13]. The carbapenems have been the drug of choice against *Acinetobacter spp.*, but the number of isolates resistant to these antimicrobial agents has increased considerably. Carbapenem resistance in *Acinetobacter spp* is associated with a variety of combined mechanisms, including the acquiring of β-lactamases, stable expression of AmpC, decreased in-membrane permeability, alteration of penicillin binding proteins, and overexpression of efflux

pumps. Among the acquired β-lactamases, enzymes of Ambler class B, also called metallo β-lactamase (MBL), and class D that hydrolyze carbapenems are the most globally identified carbapenem-resistant strains of *Acinetobacter spp* [14,15].

Currently, *Acinetobacter spp.* has developed resistance to almost all known antibiotics, and the MDR has been widely documented [16]. On the other hand, the emergence and widespread of antibiotic resistance have diminished the options of effective therapeutic drug for *Acinetobacter* infection, and a clinician has to choose the previously abandoned antibiotic colistin, which is generally associated with more serious adverse effect [17]. Most importantly, it was reported that clinical isolates resistant to colistin have emerged in certain geographical areas making the last resort of antibiotics in human medicine ineffective [18].

To address this problem effectively, knowledge about prevalence and antimicrobial resistance profile of *Acinetobacter sp.* in selective hospital's intensive care units of Khartoum state, Sudan is essential.

In the literature, many reports have shown that *Acinetobacter spp.* rapidly develops resistance to antimicrobials, and multidrug-resistant strains have been isolated [19].

Regarding Sudan, some reports are available about the prevalence of *Acinetobacter spp.* in hospitals, for example, a prevalence of 9.5% was reported in private hospital in Khartoum 2015 [20] while 30% was reported in study held in selected hospital at Khartoum state 2015 [21] and among Islamic Republic of Iran, for example, a prevalence of 15% was reported [22], in Morocco it was 9.6% [23], in India it was 9.5% [24], and in Kuwait it was 22.1% [25] In one study carried out in Saudi Arabia, Acinetobacter was the most common isolated organism among Gram-negative bacteria, with a prevalence of 31.7% [26].

2. Material and Methods

2.1. Study Design and Bacterial Isolates

This cross sectional study was carried out in three selected hospitals Khartoum state, Sudan 2018. Consecutive, non-duplicate 340 gram negative bacteria were recovered from various clinical specimens, namely; sputum, urine, wound swab, blood, CVC Tip, CSF, and bed sore swab.

The samples were collected and processed during the course of routine diagnostic work up from patients in the ICU.

The specimens received in the laboratory were inoculated on 5% Blood Agar and MacConkey Agar and incubated overnight aerobically at 37°C. Blood specimens have been inoculated on (Hi-Media, Mumbai) tryptone soya broth and then sub cultured on MacConkey agar and chocolate blood agar. Out of them, (77) isolates of *Acinetobacter* were initially identified by colonial morphology, Gram staining, growth at 37°C, a negative oxidase test, and red Kligler Iron Agar with no gas or H_2S production. API E20 (BioMe'rieux, Marcy l'Etoile, France) were used to confirm the identification of the isolates [27].

2.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was done by disc diffusion method as per the (CLSI) guidelines [28], using Muller- Hinton agar (Hi-Media, Mumbai) and antimicrobial discs (bioanalyse, Turkey and Hi-Media, Mumbai). The following antimicrobial agents (pg) were used: Ceftazidim, cefepime, cefuroxime, gentamicin, amikacin, ciprofloxacin, amoxiclav, meropenem, cephalexin, ceftriaxone aztreonam and colistin. The diameter of inhibition zones was measured and data were reported as susceptible and resistant). The quality of the disks has been checked by using reference strains.

2.3. Molecular Identification

For more confirmation of the genus PCR was performed by amplifying a fragment of 16S rDNA using these primers specific for the genus *Acinetobacter*. Acin16SF (5'-CCT TGA TGC AGA GYT AAT GC-3') and Acin16SR (5'-GTA GCA ACC CTT TGT ACC GA-3').

The PCR Amplification reactions has been performed in mixtures containing 3.5mM MgCl2, 1 unit of Taq DNA polymerase, 1µM of each primer, 0.2mM dNTPs, 1x reaction buffer of Taq DNA polymerase, and 100ng of bacterial DNA in a final volume of 30µL.

The amplification conditions with Thermal cycle as follows: an initial cycle of denaturation at 95o C for 5min followed by 25 cycles of denaturation at 95o C for 1min, annealing at 52°C for 1min and extension at 72o C for 1min, and a final extension cycle of 72o C for 8 min [29].

2.4. Statistical Analysis

The statistical analysis was performed using the SPSS Statistics version 21. The results prevented as frequency and percentage. Descriptive statistics were used to describe the main feature of study population. The Chi 2 test was obtained to study the association between Acinetobacter infection and study variables (Age, gender and types of sample). The p values less than 0.05 were considered statistically significant.

3. Result

During the study period, 77 clinical isolates of *Acinetobacter* were collected, representing 7.9% of all bacterial isolates (n=980) and 22.6% of all Gram-negative bacilli (n=340) from intensive care units. These isolates were obtained from 77 *Acinetobacter* infected patients of which 72.7% (56 cases) were males, while 27.3% (21 cases) were female, so a sex ratio M/F is 2:1 as shown in (Table 1).

Table 1. Gender distribution

Gender	*Acinetobacter*		Total
	Positive	Negative	
Male	56 (72.7%)	192 (73.0%)	248 (73.0%)
Female	21 (27.3%)	71 (27.0%)	92 (27.0%)
Total	77 (100.0%)	263 (100.0%)	340 (100.0%)

P-value 0.527 Chi-square value 0.002.

The median age of *Acinetobacter* infected patients was 53.5 years (interquartile range: 42-68 years) and the distribution by age showed that 75.3% of the isolates came from patients aged between 51-70 years; 23.4% aged 31-50 years and 1% of patients ≤ 30 years as shown in (Table 2).

Table 2. Age distribution

Age	*Acinetobacter*		Total
	Positive	Negative	
20-30 Years	1 (%)	0 (0.0%)	1 (0.1%)
31-50 Years	18 (23.4%)	73 (27.8%)	91 (26.9%)
51-70 Years	58 (75.3%)	190 (72.2%)	248 (73.0%)
Total	77 (100.0%)	263 (100.0%)	340 (100.0%)

P-value 0.002 Chi-square value 12.185.

The isolates of *Acinetobacter sp.* were obtained from various biological samples, as shown in (Figure 1).

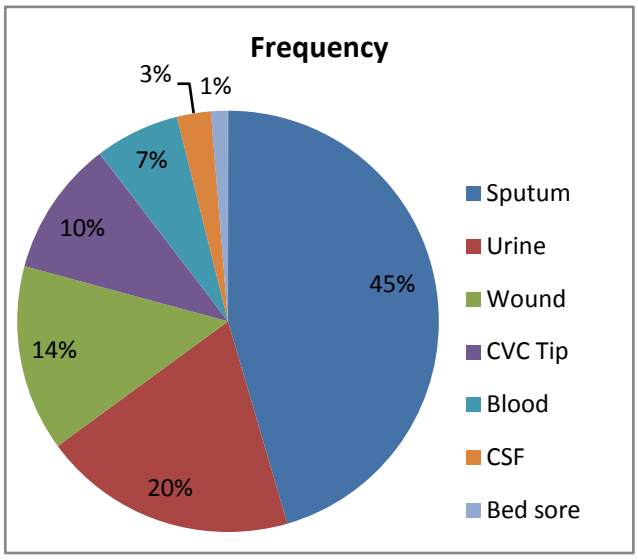

Figure 1. Biological site sampling distribution

In this study all of *Acinetobacter sp.* isolates were considered MDR as they were completely resistant to the tested antibiotics (Ceftazidim, cefepime, cefuroxime, gentamicin, amikacin, ciprofloxacin, amoxiclav, meropenem, cephalexin, ceftriaxone and aztreonam) although 100% of them were colistin sensitive.

4. Discussion

The present study shows that the infection with *Acinetobacter spp.* prevalence in Sudan is high with higher rates in ICUs. The isolation rate of *Acinetobacter* in the various samples was 22.6%. These results are within medium range compared to those from the studies conducted in private hospitals in Khartoum 2015 [20,21] where the isolation rate of *Acinetobacter* species was 9.5%, 30% respectively. The rate of prevalence of *Acinetobacter* infections in ICUs in an international studies in 3 countries (15%, 9.6%, and 9.5%) is lower than our results [22,24,26] while in other study it is equal (22.1%) [25] Whereas it is significantly higher (31.7%) than ours in another study [26]. These clinical isolates represented 7.9% of all bacterial isolates (n=980) and 22.6% of all Gram-negative bacilli (n=340) from intensive care units. The high prevalence observed in our study is probably related to non-compliance with the recommendations for mastery the hospital environment [30], lack in hands hygiene and misuse of antibiotics [31]. Some reports proven that this *Acinetobacter* which has emerged worldwide as a pathogen causing serious nosocomial infections has the ability to resist in the environment for a very long time, colonize patients or healthy subjects and can develop into an active infection at any time [32]. Since hand transmission is a major factor in the spread of this pathogen' hand hygiene and disinfection of hospital's equipment/environment are the two most important ways to control the outbreak of an epidemic *Acinetobacter* [33].

In our study, 72.7% of affected patients were male. The predominance of male patients infected with *Acinetobacter* has been verified in other studies but the reason is not justified [33,34,35,36,37]. The average age of patients in our study was 53.5 years (interquartile range: 42-68 years) with predominance of patients aged between 51-70 years; these results are similar to those of many authors [33,34,35,36,37]. The old age of patients was recognized as an independent risk factor of the acquisition of *Acinetobacter* infection [36]. Many authors have reported the predominance of *Acinetobacter* strains in Broncho-pulmonary samples [37,38,39].

In general, the *Acinetobacter* isolates are known for their resistance to various antibiotics despite their weak virulence limiting the control and infections treatment due to these microorganisms [40,41,42,43,44]. Our study shows that the rate of antibiotic resistance in Sudan is generally high and variable. Several authors have confirmed the high prevalence of these infections associated with high resistance in ICUs [37,38,45,46]. The existence of several risk factors associated with *Acinetobacter* infection such as immunocompromised persons, longer duration of stay in hospitals, invasive devices use on patients, the broad spectrum antibiotics therapy, possible and frequent contaminations and cross transmission of this bacteria through environmental reservoirs and hands of healthcare workers are the main reasons of high resistance of this microorganism in ICUs [37,32].

For the beta-lactam antibiotics which are a large family playing an important role in antimicrobial treatment, the high resistance of Acinetobacter clinical isolates to this class of antibiotics (Ceftazidim, cefepime, cefuroxime, gentamicin, amikacin, ciprofloxacin, amoxiclav, meropenem, cephalexin, ceftriaxone and aztreonam) has been described in the literature [21,46], In our study, the resistance rate against all tested beta-lactam antibiotics was 100% except in colistin which was 100% sensitive.

5. Conclusion

Acinetobacter is an important opportunistic pathogen that has a considerable, capacity to acquire mechanisms conferring resistance to a wide range of antimicrobial drugs. In this study, we showed that the prevalence of MDR *Acinetobacter* infection in ICUs of the selected hospitals in Khartoum, Sudan is high and could pose a real problem and a management impasse.

Competing Interests

The authors declare that they have no competing interests.

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