

Phenotypic Detection of Extended-Spectrum β -lactamases (ESBLs) and Aminopenicillin Cephalosporinase (AmpC)-Producing Bacterial Isolates from Surfaces of Hospital Fomites and Hands of Healthcare Workers

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ABSTRACT

Introduction: The hospital environment can significantly contribute to the spreading of bacterial isolates that pose a risk to public health. In this study, we analyzed bacteria found on hospital fomites and the hands of healthcare workers to determine the presence of resistant enzymes such as ESBLs and AmpC. **Methods:** We studied 100 samples collected from hospital fomites - including the hands of healthcare workers - for bacterial growth, which were subsequently identified using standard procedures. Standard disk methods were used to screen Gram-negative bacteria (GNB) for ESBL and AmpC production, including presumptive and confirmatory testing. **Results:** 46 (46.0%) Gram-negative bacteria were isolated from all sampling sites, including a preponderance of *Pseudomonas aeruginosa* and *Escherichia coli*. Of the 46 GNBs, 31 (67.4%) and 27 (58.7%) were resistant to ceftazidime and ceftriaxone, respectively. The double disk synergy test (DDST) showed ESBL in 34 (73.1%) of the isolates, with the highest prevalence in *E. coli* (32.3%) and *P. aeruginosa* (26.5%). These isolates were primarily associated with patients' bedding (32.4%), tablets (26.5%), and sinks (20.6%), although there was no statistical difference ($P=0.998$). Presumptive AmpC production was 100% in isolates of *K. pneumoniae*, *C. diversus*, *Shigella* spp., and *S. marcescens* but variable in other isolates. The combined disk test (CDT) showed that 29 (63.0%) isolates were AmpC-producing GNB, with the highest prevalence in *E. coli* (34.5%). **Conclusion:** The isolation of bacteria with these types of resistance from the surfaces of hospital fomites may negatively impact the quality of healthcare delivery.

INTRODUCTION

Hospital wards and associated fomites can serve as a pathway for disseminating organisms with multidrug-resistant phenotypes, particularly in developing countries with deficient infection control measures. Poor sanitation and inadequate or absent surveillance can often contribute to the spreading and acquiring of this type of bacteria within the community [1].

The increasing resistance to β -lactams by Gram-negative bacteria constitutes a problem for debilitating patients and may institute a health risk to healthcare workers and the public. This is because β -lactams are often recommended for treatment against difficult-to-treat

infections involving these organisms [2], as they can produce the desired result with low side effects [3].

The excessive use of this class of antibiotics in our healthcare facilities and communities, either due to constant recommendations or inappropriate use, has led to selective pressure and the emergence of β -lactamases, particularly extended-spectrum β -lactamases (ESBLs). Many species of bacteria employ these enzymes to reduce their susceptibility to β -lactams [4]. The ESBLs are synthesized by numerous bacteria, particularly species of Enterobacteriaceae and *Pseudomonas* [5], as well as many other Gram-negative bacteria [6]. ESBLs are typically carried on mobile genetic elements such as plasmids and

can effectively neutralize the effects of penicillin, cephalosporins, and monobactams. This renders these antibiotics ineffective against the organisms [7]. Moreover, plasmids carrying EBSLs may also harbor genes conferring resistance to other classes of antibiotics [8]. This phenomenon can limit the therapeutic options for ESBL-producing organisms and facilitate the spread of ESBLs among organisms of the same or different species [9], thereby promoting the spread of multidrug resistance (MDR) traits among bacterial species globally.

Aminopenicillin cephalosporins (AmpC) mediate resistance to 1st and 2nd-generation cephalosporins, while ESBLs mediate resistance to 3rd and 4th-generation cephalosporins. Consequently, treatment choices for common infections caused by bacterial isolates may be limited.

In recent years, the burden of enzymes promoting MDR phenotypes among bacterial isolates in our hospitals and communities has increased, partly due to poor antibiotic stewardship and surveillance systems. As such, there is a need for routine checking for these enzymes to mitigate their impact. The conventional susceptibility testing methods in our healthcare facilities may not be able to detect bacteria producing these enzymes. This can eventually lead to inappropriate diagnoses, unsuccessful therapy of patients, and unnecessary use of drugs.

The main objective of this study was to detect ESBLs phenotypically and AmpC in isolates of *Enterobacteriaceae*, and *P. aeruginosa* recovered from hospital fomites and the hands of healthcare workers for epidemiological purposes. By identifying these resistance mechanisms, we can work to limit their spread.

MATERIAL AND METHODS

Study area. The study was conducted in Mubi General Hospital, located in Mubi-South LGA of Adamawa State, at coordinates 10°15'54.9"N 13°16'10.0"E.

Sample collection. We randomly collected 100 non-clinical samples from various locations within the wards of Mubi General Hospital, including sinks, bedding, tables, door handles, and the hands of healthcare workers. The samples were collected using sterile swab sticks, and each was immediately introduced into MacConkey agar, Eosin Methylene Blue agar, Cetrimide agar, and Salmonella-Shigella agar. The agar plates were incubated aerobically at 37°C for 24 h. Then, the pure isolates were aseptically transferred into nutrient agar slants and refrigerated at 4 °C for further use.

Bacteria identification. After Gram-staining, bacteria isolates were identified phenotypically on a Microgen A kit [10]. However, *P. aeruginosa* isolates were identified based on their reaction to the cetrimide agar plate.

Phenotypic detection of ESBL

Presumptive test. The bacteria isolates were investigated for susceptibility to third-generation

cephalosporins using ceftazidime (30µg) and ceftriaxone (30µg) antibiotic discs (Oxoid, UK). The bacterial strain with zones of inhibition of ≤ 22 mm for ceftazidime and ≤ 25 mm for ceftriaxone were deemed to be likely ESBL-producing organisms [11].

Confirmatory test. Bacterial isolates resistant to third-generation cephalosporin were subjected to confirmatory tests using the double disc synergy test (DDST). A bacterial suspension corresponding to a 0.5% MacFarland standard was introduced onto a Mueller-Hinton agar (MHA) plate for each test. Antibiotic discs of amoxicillin/clavulanic acid (30 µg), ceftazidime (30 µg), and ceftriaxone (30 µg) were positioned 15 mm apart from each other on separate MHA plates and left to incubate for 18-24 h at 35-37 °C. The isolate that displayed a distinct enlargement of the ceftazidime or ceftriaxone inhibition zone towards the disc containing clavulanate was considered an ESBL-producing organism [11].

Presumptive AmpC beta-lactamase detection. To detect presumptive AmpC beta-lactamase, the bacteria were tested with 30 µg cefoxitin discs and isolates with a diameter zone of inhibition ≤ 18 mm were defined as AmpC-producing [12].

Confirmatory AmpC β-lactamase production. To confirm AmpC enzyme production, ceftazidime (30 µg) and cefotaxime (30 µg) discs were positioned 20 mm away from the cefoxitin (30 µg) disc on the MHA plate that was already seeded with the test isolate. Confirmatory AmpC enzyme production was considered when there was an increase in the diameter of the zone of inhibition by ≥ 5 mm towards either of the cephalosporins (ceftazidime or cefotaxime) used in combination with the cefoxitin disc. Furthermore, bacterial isolates that were AmpC-positive displayed a blunting of the ceftazidime or cefotaxime zone of inhibition adjacent to the cefoxitin disk [13].

Statistical analysis. All the data obtained were presented as percentages. One-way analysis of variance (ANOVA) was used to determine the significance level in all the data obtained for ESBLs and AmpC. All statistical analysis was done using IBM SPSS Statistics version 21 (Armonk, NY: IBM Corp).

Ethical considerations. The management and participating healthcare workers of the hospital where the study was carried out were informed of the goal and objectives of the study, and consent was obtained from them all. The research was approved by the seminar and research committee of the Department of Biological Science Technology Federal Polytechnic Mubi, Adamawa State, Nigeria, with the reference number FPM/BST/SRC/Vol.1/2022.105.

RESULTS

Our research indicates that Gram-negative bacteria (GNB) are prevalent in contaminated hospital fomites and the hands of healthcare workers. Specifically, we found

that *P. aeruginosa* and *E. coli* were the most prominent GNB species detected across all sampling sites (Fig. 1).

Table 1 displays the susceptibility of GNB to ceftazidime and ceftriaxone, which were used as markers to identify Extended-Spectrum β -lactamase (ESBL) producing GNB. Of the 46 GNBs tested, 31 (67.4%) and

27 (58.7%) were resistant to ceftazidime and ceftriaxone, respectively. Notably, all the isolates of *K. pneumoniae*, *C. diversus*, *Shigella* spp., and *S. marcescens* were resistant to both antibiotics. These findings determined that all isolates exhibiting resistance to ceftazidime and ceftriaxone should be considered presumptive ESBL-producing organisms.

Table 1. Frequency (%) of Gram-negative bacteria resistant to beta-lactam antibiotics

Isolates	No (%)	Ceftazidime	Ceftriaxone
<i>Escherichia coli</i>	15 (15.0)	11 (73.3)	10 (66.7)
<i>Klebsiella pneumoniae</i>	3 (3.0)	3 (100)	3 (100)
<i>Pseudomonas aeruginosa</i>	16 (16.0)	7 (43.8)	4 (25.0)
<i>Citrobacter diversus</i>	4 (4.0)	4 (100)	4 (100)
<i>Shigella</i> spp	2 (2.0)	2 (100)	2 (100)
<i>Providencia rettgeri</i>	4 (4.0)	2 (50.0)	2 (50.0)
<i>Serratia marcescens</i>	2 (2.0)	2 (100)	2 (100)

Table 2. Prevalence (%) of ESBL-producing Gram-negative bacteria from the hospital environment

Isolates	Sink ^a	Table ^a	Beddings ^a	Door handle ^a	Hands HCW ^a	of	Total
<i>Escherichia coli</i>	1 (14.3%)	5 (55.6%)	3 (27.3%)	2 (50.0%)	-		11 (32.4%)
<i>Klebsiella pneumoniae</i>	-	1 (11.1%)	2 (18.2%)	-	-		3 (8.8%)
<i>Pseudomonas aeruginosa</i>	4 (57.1%)	1 (11.1%)	2 (18.2%)	1 (25.0%)	1 (33.3%)		9 (26.5%)
<i>Citrobacter diversus</i>	-	-	2 (18.2%)	1 (25.0%)	1 (33.3%)		4 (11.8%)
<i>Shigella</i> spp	-	-	1 (9.1%)	-	1 (33.3%)		2 (5.9%)
<i>Providencia rettgeri</i>	2 (28.6%)	1 (11.1%)	-	-	-		3 (8.8%)
<i>Serratia marcescens</i>	-	1 (11.1%)	1 (9.1%)	-	-		2 (8.8%)
Total	7 (20.6%)	9 (26.5%)	11 (32.4%)	4 (11.8%)	3 (8.8%)		34 (72.3%)

Legend: Parameter with the same superscript suggest a lack of significant difference ($P=0.998$).

Our findings, confirmed by DDST (Figure 2), indicate that ESBL production was detected in 34 out of 46 isolates, resulting in an overall prevalence of 73.9%. Notably, we observed a higher prevalence of ESBL production in *E. coli* (32.3%) and *P. aeruginosa* (26.5%)

isolates. We found that Extended-Spectrum β -lactamase-producing GNB were most commonly present on patient's bedding (32.4%), tables used by healthcare workers (26.5%), and sinks (20.6%), but with no statistical difference ($P=0.998$) as shown in Table 3.

Table 3. Frequency (%) of Gram-negative bacteria resistant to cefoxitin

Isolates	No (%)	FOX (%)
<i>Escherichia coli</i>	15 (15.0)	10 (66.7)
<i>Klebsiella pneumoniae</i>	3 (3.0)	3 (100)
<i>Pseudomonas aeruginosa</i>	16 (16.0)	7 (43.8)
<i>Citrobacter diversus</i>	4 (4.0)	4 (100)
<i>Shigella</i> spp	2 (2.0)	2 (100)
<i>Providencia rettgeri</i>	4 (4.0)	2 (50.0)
<i>Serratia marcescens</i>	2 (2.0)	2 (100)
Total	46 (46.0)	30 (65.2)

Legend: FOX= cefoxitin

Table 3 also provides the results for presumptive AmpC-producing GNB. All the *K. pneumoniae*, *C. diversus*, *Shigella* spp., and *S. marcescens* isolates were resistant to cefoxitin, while other bacterial isolates showed variable resistance to the antibiotic.

Table 4 confirms that 29 out of 46 isolates were AmpC-producing GNB, with an overall prevalence of 63.0%. Our results indicate that AmpC-producing isolates were most prevalent among *E. coli* (34.5%) and *P. aeruginosa* (17.2%), while *S. marcescens* and *Shigella* spp. had the

lowest prevalence, at 6.9%. Our findings also reveal that AmpC-producing GNB was predominantly recovered from tables and beddings with a prevalence rate of 32.1%. In contrast, AmpC-producing GNB were least frequently recovered from door handles, with a prevalence rate of 3.6%. However, statistical analysis indicates no significant difference in prevalence rates between these locations ($P=0.999$).

Table 4. Prevalence (%) of AmpC-producing Gram-negative bacteria from the hospital environment

Isolates	No. of isolates	Sink ^a (%)	Table ^a (%)	Beddings ^a (%)	Door handle ^a (%)	Hands of HCW ^a (%)	Total (%)
<i>Escherichia coli</i>	15	1 (25.0)	5 (55.6)	2 (22.2)	-	2 (40.0)	10 (35.7)
<i>Klebsiella pneumoniae</i>	3	-	1 (11.1)	2 (22.2)	-	-	3 (10.7)
<i>Pseudomonas aeruginosa</i>	16	2 (50.0)	1 (11.1)	1 (11.1)	-	1 (20.0)	5 (17.9)
<i>Citrobacter diversus</i>	4	-	-	2 (22.2)	1 (100)	1 (20.0)	4 (14.3)
<i>Shigella</i> spp	2	-	-	1 (11.1)	-	1 (20.0)	2 (7.1)
<i>Providencia rettgeri</i>	4	1 (25.0)	1 (11.1)	-	-	-	2 (7.1)
<i>Serratia marcescens</i>	2	-	1 (11.1)	1 (11.1)	-	-	2 (7.1)
Total	46 (63.9%)	4 (14.3)	9 (32.1)	9 (32.1)	1 (3.6)	5 (17.9)	28 (59.6)

Legend: Parameters with the same superscript suggest a lack of significant difference ($P=0.998$). HCW=healthcare workers

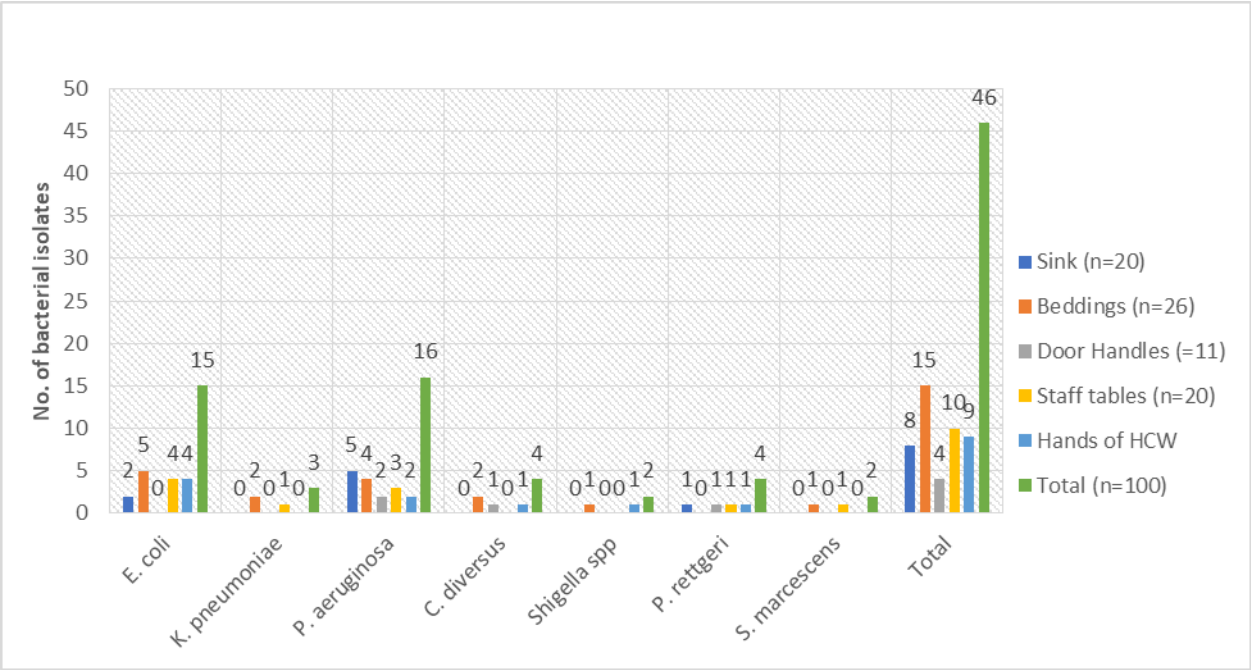


Fig. 1. Frequency of Gram-negative bacteria on surfaces of hospital fomites and hands of healthcare workers.

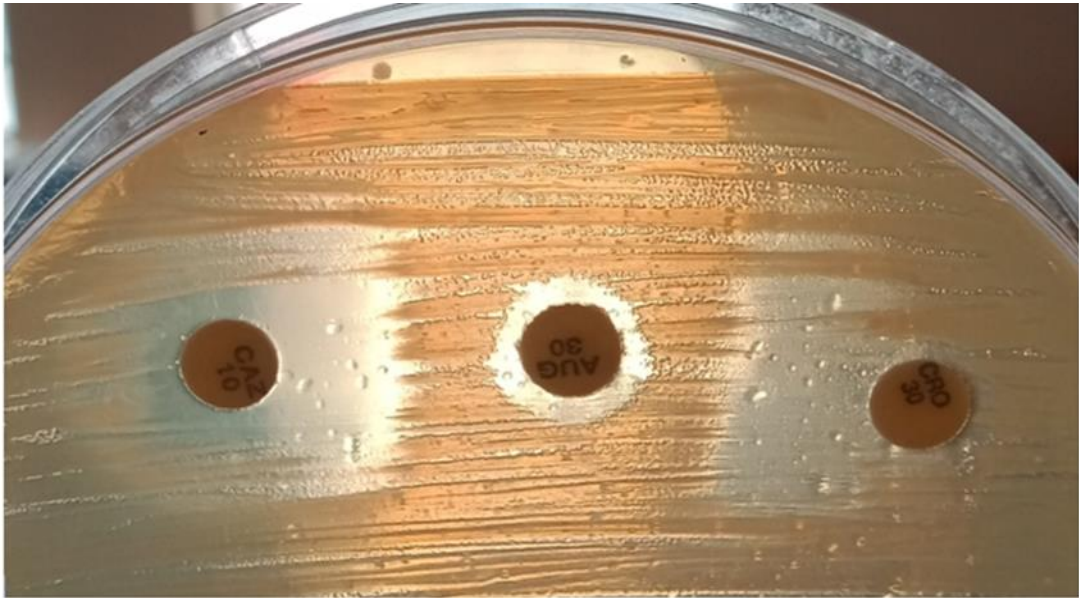


Fig. 2. ESBL Positive (DDST) plate on Mueller-Hinton agar

DISCUSSION

The results of our study are significant in that we detected high levels of antibiotic-resistant Gram-negative bacteria (GNB) on hospital fomites and the hands of healthcare workers. These findings highlight the potential role of fomites and human contact in transmitting antibiotic-resistant GNB within healthcare settings. This finding supports the well-established notion that GNBs, such as *E. coli*, *P. aeruginosa*, and *Klebsiella pneumoniae*, are responsible for nosocomial infection, especially among patients with extended hospitalization [14, 15].

Our study used two combinations of cephalosporin disks in conjunction with an amoxicillin-clavulanic acid disc to detect ESBL-producing organisms. Of the two antibiotic disks, we found that ceftazidime was the most effective ESBL detector for *E. coli*. In contrast, combining ceftazidime and ceftriaxone with amoxicillin-clavulanic acid was the most effective in detecting ESBL-producing *K. pneumoniae*, *Citrobacter diversus*, *Shigella* spp., and *Serratia marcescens*. This observation is consistent with the findings of a previous study [16], which suggests that using multiple disk combinations may be necessary to detect ESBL production accurately. Failing to do so could lead to underreporting of prevalence rates. To screen for ESBL-producing organisms effectively, we recommend simultaneously using two or more cephalosporin disks. Furthermore, all *Enterobacteriaceae* strains resistant to cefoxitin also tested positive for AmpC production in the present study.

A study conducted in Brazil on Gram-negative bacteria recovered from various surfaces in a neonatal intensive care unit reported a prevalence rate of 63.3% for AmpC-producing GNB among the isolated bacteria, which is quite similar to the finding of our study. However, it is worth noting that the same survey reported a prevalence rate of 33.3% for ESBL-producing GNBs, which contrasts our study results [17]. Studies in Brazil have reported lower prevalence rates of ESBL-producing GNBs from contaminated hospital surfaces, e.g., 15.2% [15] and 24.8% [18]. Another study from Algeria [16] reported a prevalence rate of 21.4% for ESBL among GNBs isolated from the hospital environment. The disparity in the prevalence rates between our study and others may be attributed to a variety of factors, such as the differences in the frequency and adherence to decontamination/disinfection procedures within hospital environments, variations in sample size, differences in socio-cultural backgrounds, and geographical location, among other factors.

ESBLs have emerged as a leading public health concern in nosocomial infections associated with *Enterobacteriaceae*. They are widely disseminated worldwide and reported in developing and developed countries [15, 19]. Numerous studies have postulated that various factors, such as healthcare processes or facilities and commonly-touched surfaces, among others, may

serve as risk factors for acquiring and being infected with ESBL-producing GNBs [16, 20, 21].

In the present study, ESBL and AmpC-producing GNBs were mainly associated with beddings, tables, and sinks. The high rate of these organisms contaminating beddings may be attributed to constant contact with patients and health care workers. A previous study reported that contamination levels on hospital bedding ranged from 10^2 to more than 10^5 cfu/10 cm² after just one night of use [22]. In addition to constant contact with patients and healthcare workers, hospital bedding may also become contaminated due to the use of whole or broken hospital mattresses. A study conducted in the United States of America reported that terminal cleaning failed to eliminate bacteria from the surface of the mattress. Another study indicated that hospital mattresses are often the most heavily contaminated areas within hospital rooms, especially when they are ruptured, soiled, or contaminated with infected exudates from patients. If not replaced, such mattresses have the potential to contaminate the bedding used on them [23, 24].

Our findings were in contrast to those of a previous study that correlated the detection of ESBL strains with work surfaces, toilet seats, and incubators [16]. Such a disparity may be attributed to differences in the types of inanimate surfaces employed in both studies. The absence of significant difference in the detection of ESBL and AmpC-producing GNBs on all the surfaces and hands of healthcare workers suggests that all surfaces may become contaminated at an equal rate, depending on the source of contamination. According to recent reports, *E. coli* and *Klebsiella* spp. may survive for more than a year in dry surroundings, while *S. marcescens* can survive up to two months [16, 25]. The high detection of ESBL and AmpC-producing *P. aeruginosa* in sinks could be due to the consistently damp environment they provide. Previous studies have shown that *P. aeruginosa* is often isolated in moist environments where it can form biofilms [26, 27].

The most prevalent ESBL-producing Gram-negative bacteria detected in the current study was *E. coli*, corroborating a previous report from Gaza, Palestine [28]. Unlike the finding of this study, several other studies across Africa have reported a higher prevalence of *K. pneumoniae* over *E. coli*, including studies from Sudan [29], Ethiopia [30], Algeria [16], and Zimbabwe [31].

According to the World Health Organization (WHO), patient safety is the absence of damage throughout the care process. In the context of our study, the detection of GNB with ESBL and AmpC-producing potentials could pose a higher risk for hospital patients who are already immunocompromised and increase the risk of treatment failure. This is because potential ESBL-producing organisms may carry mobile genetic elements capable of transferring or acquiring other resistance genes with grave consequences [15, 32, 33].

The presence of resistant bacteria in hospitals represents a severe risk to the health and recovery of patients who require care in these facilities. Healthcare professionals must be aware of the possible sources of contamination in the hospital environment to establish infection control measures that can help reduce infections and improve patient survival rates.

We isolated ESBL and AmpC-producing Gram-negative bacteria from the hands of healthcare workers and inanimate surfaces in the hospital environment, particularly from the bedding. Most of the ESBL and AmpC-producing GNB isolated in our study were *E. coli*. The presence of bacterial isolates with these resistance traits on surfaces in close contact with the patient may disrupt quality healthcare delivery, increase the burden of antibiotic resistance, prolong hospital stay, and significantly contribute to treatment failure in the hospital.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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