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Original Article **Open Access**

Environmental contamination rates by extended-spectrum β-lactamase producing *Acinetobacter baumannii* in selected hospitals in southwest Nigeria

Akinbobola Ayorinde

Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria
Correspondence to: ayorinde.akinbobola@aaua.edu.ng

Abstract:

Background: *Acinetobacter baumannii* poses significant threat in healthcare facilities due to its innate ability to persist on harsh environmental surfaces for extended periods and its common resistance to multiple antibiotics, through mechanisms such as extended-spectrum β-lactamase (ESBL) production. Healthcare-associated (HAI) *A. baumannii* infections are a growing concern in low-and-middle-income countries (LMICs), with environmental surfaces serving as important reservoirs for transmission. However, there is limited information on *A. baumannii* contamination rates of environmental surfaces in healthcare facilities. This study determined the contamination rates and antimicrobial susceptibility profiles of *A. baumannii* from high-touch surfaces in selected hospitals in Akoko area of Ondo State, southwest Nigeria.
Methodology: A total of 204 environmental samples were collected by swabbing selected surfaces (48 door-knobs, 120 bedframes, and 36 handwashing basins) of 4 selected hospitals (hospital A, B, C and D). Samples were cultured on MacConkey agar plates and incubated aerobically at 37°C for 24 hours. Colonies isolated from the samples were identified as *A. baumannii* based on cultural morphology and conventional biochemical test scheme. The susceptibility of the isolates to selected antibiotics was determined by the Kirby Bauer disc diffusion method in line with the Clinical and Laboratory Standards Institute (CLSI) guidelines, and ESBL production in selected isolates was detected by the double disk synergy test (DDST). Multi-drug resistance (MDR) in an isolate was defined as resistance to three or more antibiotic categories. Differences in contamination rates between the hospital sites and touch surfaces were compared using Pearson's Chi squared test, with $p<0.05$ considered as statistical significance.
Results: *Acinetobacter baumannii* was isolated from 13.7% (28/204) of the environmental samples, with the highest contamination rate recorded for samples collected from handwashing basins (33.3%, 12/36) while bed frames had the lowest *A. baumannii* contamination rate (4.2%, 5/120) ($x^2=24.372$, $p<0.0001$). Hospital site D had the highest *A. baumannii* contamination rate of 19.5% (10/51) while hospital site A had the lowest rate of 7.8% (4/51) ($x^2=4.305$, $p=0.2303$). The *A. baumannii* isolates were commonly resistant to the third-generation cephalosporins (39.3% to ceftazidime, 32.1% to ceftriaxone and 28.5% to cefotaxime), with 7 of 11 (63.6%) cephalosporin-resistant isolates positive for ESBL production. Four (14.3%) *A. baumannii* isolates were MDR. `
Conclusion: The isolation ESBL-producing and MDR *A. baumannii* from environmental surfaces in the selected hospitals in this study underscores the importance of stringent infection control practices and environmental surveillance in resource-limited settings to ensure patient safety.

Keywords: *Acinetobacter baumannii*, ESBL, MDR, high-touch surface, hospital, contamination

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Taux de contamination environnementale par *Acinetobacter baumannii*, producteur de β-lactamases à spectre étendu, dans certains hôpitaux du sud-ouest du Nigéria

Akinbobola Ayorinde

Département de Microbiologie, Université Adekunle Ajasin, Akungba-Akoko, État d'Ondo, Nigéria.
Correspondance: ayorinde.akinbobola@aaua.edu.ng

Résumé:

Contexte: *Acinetobacter baumannii* représente une menace importante dans les établissements de santé en raison de sa capacité innée à persister sur des surfaces environnementales difficiles pendant de longues périodes et de sa résistance fréquente à de multiples antibiotiques, grâce à des mécanismes tels que la production de β-lactamases à spectre étendu (BLSE). Les infections nosocomiales à *A. baumannii* constituent une préoccupation croissante dans les pays à revenu faible ou intermédiaire (PRFI), les surfaces environnementales constituant d'importants réservoirs de transmission. Cependant, les informations sur les taux de contamination par A. baumannii des surfaces environnementales des établissements de santé sont limitées. Cette étude a déterminé les taux de contamination et les profils de sensibilité aux antimicrobiens d'*A. baumannii* sur des surfaces fréquemment touchées dans des hôpitaux sélectionnés de la région d'Akoko, dans l'État d'Ondo, au sud-ouest du Nigéria.
Méthodologie: Au total, 204 échantillons environnementaux ont été prélevés par écouvillonnage de surfaces sélectionnées (48 poignées de porte, 120 cadres de lit et 36 lavabos) dans quatre hôpitaux sélectionnés (hôpitaux A, B, C et D). Les échantillons ont été cultivés sur gélose MacConkey et incubés en aérobiose à 37°C pendant 24 heures. Les colonies isolées des échantillons ont été identifiées comme appartenant à *A. baumannii* sur la base de leur morphologie et d'un protocole de tests biochimiques conventionnels. La sensibilité des isolats aux antibiotiques sélectionnés a été déterminée par la méthode de diffusion sur disque de Kirby Bauer, conformément aux directives de l'Institut des normes cliniques et de laboratoire (CLSI), et la production de BLSE dans les isolats sélectionnés a été détectée par le test de synergie à double disque (DDST). Français La multirésistance aux antibiotiques (MDR) dans un isolat a été définie comme une résistance à trois catégories d'antibiotiques ou plus. Les différences de taux de contamination entre les sites hospitaliers et les surfaces de contact ont été comparées à l'aide du test du Chi carré de Pearson, avec $p<0,05$ considéré comme statistiquement significatif.
Résultats: *Acinetobacter baumannii* a été isolé de 13,7% (28/204) des échantillons environnementaux, le taux de contamination le plus élevé étant enregistré pour les échantillons prélevés dans les lavabos (33,3%, 12/36) tandis que les cadres de lit présentaient le taux de contamination par *A. baumannii* le plus faible (4,2%, 5/120) ($x^2=24,372$, $p<0,0001$). Le site hospitalier D présentait le taux de contamination par *A. baumannii* le plus élevé, soit 19,5% (10/51), tandis que le site hospitalier A présentait le taux le plus faible, soit 7,8% (4/51) ($x^2=4,305$, $p=0,2303$). Les isolats d'*A. baumannii* étaient généralement résistants aux céphalosporines de troisième génération (39,3% à la céftazidime, 32,1% à la céftriaxone et 28,5% à la céfotaxime), avec 7 des 11 isolats résistants aux céphalosporines (63,6%) positifs à la production d'ESBL. Quatre isolats d'*A. baumannii* (14,3%) étaient multirésistants.
Conclusion: L'isolement d'*A. baumannii* producteurs de BLSE et multirésistants à partir de surfaces environnementales dans les hôpitaux sélectionnés dans cette étude souligne l'importance de pratiques rigoureuses de contrôle des infections et de surveillance environnementale dans les environnements à ressources limitées pour garantir la sécurité des patients.

Mots-clés: *Acinetobacter baumannii*, BLSE, multirésistant, surface fréquemment touchée, hôpital, contamination

Introduction:

Acinetobacter baumannii is an aerobic Gram-negative coccobacillus opportunistic bacterium, known to cause a wide range of infectious diseases including meningitis, urinary tract infection, bacteraemia, wound infections and pneumonia in susceptible host (1). It is a common cause of healthcare-associated infections (HAIs) with high mortality rate (2). Prolonged hospital stays, weakened immunity, overuse of broad-spectrum antibiotics, comorbidities such as diabetes mellitus and burns, invasive procedures, and presence of indwelling catheters or mechanical ventilators are common risk factors for acquiring *A. baumannii* infections (3,4).

The propensity of *A. baumannii* to develop resistance to multiple antibiotics further complicates the challenges posed by *A. bau mannii* infection. Hard-to-treat multidrug-resistant strains of *A. baumannii* have been widely reported globally prompting the World Health Organisation to designate carbapenem-resistant *A. baumannii* as one of the most critical priority pathogens for which new therapeutics are urgently needed (5).

Acinetobacter baumannii infections are mostly HAIs with community-acquired infections (CAIs) not commonly reported (6). Its ability to survive harsh environmental conditions in hospital environments and persist on surfaces for extended periods makes it a common HAI pathogen (7). Thus, environmental contamination plays a significant role in the epidemiology of healthcare-acquired *A. baumannii* infections.

Healthcare-associated infections generally constitute a significant public health challenge causing prolonged hospital stays and sometimes death (8). HAIs are major health challenge in low-and-middle-income countries (LMICs), with an estimated prevalence of 19.2% compared to 12% in high-income countries (5). The less stringent infection control measures in LMICs due to the limited resources are largely responsible for the higher prevalence of HAIs in LMICs compared to high-income countries.

The hospital environment can act as reservoir of HAI pathogens such as *A. baumannii*. Contact with contaminated surfaces is a major means of acquiring healthcare-associated *A. baumannii* infection therefore it is important to identify potential hospital surfaces that can serve as reservoirs for *A. bau-*

mannii. This is crucial in preventing the exposure of susceptible patients to *A. baumannii* infection.

Although the hospital environment has been variously implicated as the reservoir for MDR HAI pathogens, there is dearth of information on the persistence of pathogenic microorganisms in the hospitals in sub-Saharan Africa. This study investigated the contamination of surfaces by *A. baumannii* in clinical areas of selected hospitals, southwest Nigeria.

Materials and method:

Study design:

This is an environmental survey of surfaces of acute medical facilities in southwest Nigeria. Surfaces in selected publicly (sites A, B and D) and privately (site C) owned acute medical facilities were sampled for contamination with *A. baumannii*. Surfaces sampled include high-touch surfaces such as bed frames, doorknobs and hand-washing basins in the clinical areas of the selected healthcare facilities. Sampling of surfaces was done three times on different occasions.

Sampling of hospital surfaces:

Samples were collected from the selected surfaces with sterile swabs, which were labelled and moistened with sterile distilled water under aseptic conditions before use. Selected surfaces were then severally swabbed at different spots to ensure adequate coverage. Sampling of selected surfaces was done in triplicates at each sampling using different swabs. The swabs were transported on ice parks to the laboratory for enrichment and isolation of *A. baumannii.*

Isolation of *A. baumannii*:

The cotton buds from the swabs were aseptically cut and transferred into 5 ml sterile distilled water which was vigorously vortexed at maximum speed for 1 minute. Samples from the surfaces were enriched by transferring 1ml of the sample suspension into 9ml of sterile trypticase soy broth (Oxoid, UK). The inoculated broths were incubated for 24 hours at 37^{o}C, after which 200µL was inoculated onto Blood and Mac- Conkey agar plates (LAB M, UK) prepared according to the manufacturer's instructions. The inoculated plates were incubated for 24 hours at 37^{o}C.

Colonies showing cultural characteristics similar to *Acinetobacter* on MacConkey agar were sub-cultured onto fresh agar to obtain pure culture of selected colonies. The pure cultures of selected isolates were identified as *A. baumannii* based on their colony morphology, Gram staining reaction and biochemical tests including catalase, oxidase, motility, citrate, indole, methyl red, urease, and oxidation-fermentation (OF) reaction (9).

Antibiotic susceptibility test:

The susceptibility of *A. baumannii* isolates to commonly used antibiotics was determined by the Kirby-Bauer disc diffusion test. Briefly, standardised inoculum (0.5 McFarland standard) was prepared from overnight cultures of the isolates and inoculated on freshly prepared Mueller–Hinton (MH) agar (LAB M, UK) using sterile swabs. Antibiotic discs (Abteck, UK) were aseptically placed on the inoculated MH agar after 30 minutes. Antibiotics used include ceftazidime (30µg), ceftriaxone (30µg), cefotaxime (30µg), gentamicin (10 µg), meropenem (10µg), ciprofloxacin (5µg) and amikacin (30µg).

All the plates were subsequently incubated at 37°C for 18 hours after which the diameter of the zones of inhibition observed around each disc was measured and the results were interpreted as being resistant, intermediate or susceptible to the antibiotics using the CLSI guidelines (10). Isolates resistant to antibiotics belonging to three or more classes of antibiotics were classified as multidrug resistant (MDR) isolates.

Detection of extended spectrum β-lactamase (ESBL) production:

The double disc synergy test (DDST), as described by Kaur et al., (11) with slight modification, was used to phenotypically confirm ESBL production in A. *baumannii* isolates that were resistant to third-generation cephalosporins in the antibiotic susceptibility test. Briefly, cefotaxime (30µg) and ceftazidime (30µg) discs were placed at 1.5cm from the centre of amoxicillin (20 µg)/clavulanic acid (10µg) disc placed at the centre of a freshly prepared MH agar plate that have been seeded with standardised (0.5 McFarland standard) inoculum of selected *A. baumannii* isolate. The MH plate was incubated at 37^{o}C for 24 hours and isolate showing enhanced zone of inhibition around the cephalosporin discs towards the amoxicillin-clavulanic acid disc was interpreted as being positive for ESBL production.

Data analysis:

Descriptive statistic was used to describe the number of samples contaminated with *A. baumannii* and number of isolates resistant to tested antibiotics as percentages. Differences in the contamination rates between the different hospitals was compared by Pearson's Chi squared test with $p<0.05$ considered to be statistically significant.

Results:

A total of 204 environmental samples were collected from different surfaces in the 4 selected hospital sites; 12 from doorknobs, 9 from handwash basins and 30 bedframe

samples. The frequency of samples with *A. baumannii* contamination is shown in Table 1. A total of 28 isolates were identified as *A. baumannii* based on their cultural morphology, Gram staining reaction and biochemical characteristics.

Samples collected from hospital site D had the highest *A. baumannii* contamination rate (19.5%, 10/51) while hospital site A had the lowest *A. baumannii* contamination rate of 7.8% (4/51) (x^2=4.305, p=0.2303). Overall, handwashing basins had the highest *A. baumannii* contamination rate of 33.3% (12/36) while bedframes had the lowest contamination rate of 4.2% (5/120) (x^2= 24.372, p<0.0001).

The susceptibility of *A. baumannii* isolates to selected antibiotics is shown in Table 2. Most of the *A. baumannii* isolates were susceptible to amikacin (89.3%) and ciprofloxacin (85.7%) while ceftazidime (at 60.7% susceptible) was the least active against the isolates. Four (14.3%) of the 28 isolates were MDR isolates based on resistance to antibiotics in three or more classes. Seven (64.0%) of 11 *A. baumannii* isolates resistant to third-generation cephalosporins (ceftazidime) evaluated for ESBL production by DDST were confirmed to be ESBL producers (Fig 1).

Table 1: Frequency distribution of environmental samples contaminated with *Acinetobacter baumannii* in selected hospital sites

Surfaces sampled	**Hospital sampling site**				**Total (%)**
	Site A (%)	**Site B (%)**	**Site C (%)**	**Site D (%)**	
Doorknobs (n=12 each)	2 (16.7)	3 (25.0)	2 (16.7)	4 (33.3)	11 (22.9)
Wash hand basin (n=9 each)	2 (22.2)	4 (44.4)	3 (33.3)	3 (33.3)	12 (33.3)
Bed frames (n=30 each)	0	2 (6.7)	0	3 (10.0)	5 (4.2)
Total (n=51 each)	**4 (7.8)**	**9 (17.6)**	**5 (9.8)**	**10 (19.5)**	**28 (13.7)**

Table 2: Frequency of resistance in *Acinetobacter baumannii* isolated from hospital environment to selected antibiotics

Antibiotics	**Hospital sampling site**				**Total (%) (n=28)**
	Site A (%) (n=4)	**Site B (%) (n=9)**	**Site C (%) (n=5)**	**Site D (%) (n=10)**	
Ceftazidime (30µg)	2 (50.0)	3 (33.3)	2 (40.0)	4 (40.0)	**11 (39.3)**
Gentamicin (10µg)	1 (25.0)	2 (22.2)	1 (20.0)	2 (20.0)	**6 (21.4)**
Meropenem (10µg)	1 (25.0)	3 (33.3)	2 (40.0)	3 (30.0)	**9 (32.1)**
Ceftriaxone (30µg)	0	3 (33.3)	2 (40.0)	4 (40.0)	**9 (32.1)**
Cefotaxime (30µg)	1 (25.0)	3 (22.2)	2 (40.0)	3 (30.0)	**8 (28.5)**
Ciprofloxacin (5µg)	0	2 (22.2)	0	2 (20.0)	**4 (14.3)**
Amikacin (30µg)	0	1 (11.1)	1 (20.0)	1 (10.0)	**3 (10.7)**

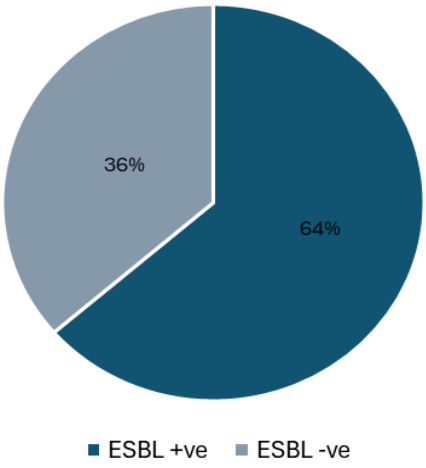

Fig 1: Frequency distribution of ESBL-producing and non-ESBL producing *Acinetobacter baumannii* isolates to third generation cephalosporins

Discussion:

Contaminated environmental surfaces have long been recognised as significant exogenous sources of a wide range of HAI pathogens, such as *A. baumannii*. The ability of *A. baumannii* to persist for extended periods on these surfaces increases its transmission potential in healthcare facilities. Non-adherence to strict infection control practices in resource-limited countries, due to various constraints, further heightens the risk of transmitting HAI pathogens from environmental reservoirs. Multi--drug resistance in *A. baumannii* complicates the management of HAI infections caused by this organism, as therapeutic options are limited. This presents a substantial challenge in resource-limited countries because of limited access to antimicrobial agents. This study determined the prevalence of ESBL-producing MDR *A. baumannii* on the surfaces of selected healthcare facilities in resource-limited settings to assess the risks of acquiring *A. baumannii* HAI in the studied area.

Acinetobacter baumannii was isolated from the selected surfaces in all the medical facilities in this study indicating common *A. baumannii* surface contamination in the medical facilities in the studied region. *A. baumannii* has been previously isolated from environmental surfaces in medical facilities in Nigeria (12,13). The potential for *A. baumannii* to persist on environmental surfaces may be attributed to the ability of *A. baumannii* to survive the ambient conditions in the region. A wide range of pathogenic organisms are known to persist for extended periods at ambient conditions in tropical regions. For instance, Akinbobola et al., (14) reported that common HAI pathogens including A. *baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa* can persist on commonly used hospital fabrics for a significant period in tropical ambient conditions. Additionally, *A. baumannii* is known to survive harsh environmental conditions due to its innate ability to tolerate desiccation and form protective biofilm biomass (15,16).

Of all the surfaces sampled in the study, handwashing basins were most contaminated by *A. baumannii.* This can be attributed to the potential of moisture to enhance the survival of *A. baumannii* on harsh environmental surfaces (14). Likewise, the hands of healthcare workers are important routes for the dissemination of HAI pathogens such as *A. baumannii* in healthcare facilities (17,18). Thus, the relatively higher contamination of handwashing basins with *A. baumannii* compared to other surfaces in this study can also be attributed to the potential shedding of *A. baumannii* from contaminated hands of healthcare workers during hand washing. Handwashing basins have been previously identified as common reservoirs of *A. baumannii* infection outbreaks in healthcare facilities (19,20). The frequent contamination of doorknobs also a high touch surface with *A. baumannii* in the healthcare facilities studied further reveal the potential for *A. baumannii* dissemination through contaminated hands of healthcare workers and patients.

The widespread resistance to antibiotics in *A. baumannii* isolates is a major challenge in the treatment of *A. baumannii* infections. *A. baumannii* strains isolated from surfaces in this study were resistant to different antibiotics tested. The common resistance to third generation cephalosporin antibiotics such as ceftazidime, cefotaxime and ceftriaxone observed in this study can be attributed to widespread ESBL production in Gram-negative bacteria like *A. baumannii* isolates (21). Most of the *A. baumannii* isolates (~64%) resistant to third-generation cephalosporins in this study produced ESBL further indicating the widespread occurrence of ESBL in *A. baumannii* isolates. This has significant implica-

tions for the treatment of *A. baumannii* infections in LMICs like Nigeria due to the limited availability of relatively expensive alternatives.

Conclusion:

In conclusion, results from this study demonstrated common contamination of hospital surfaces with MDR *A. baumannii* indicating a potential risk of transmission to susceptible patients. Common resistance to multiple antibiotics in the *A. baumannii* isolates further complicates the threat posed by the contamination of sampled surfaces in the selected healthcare facilities. This underscores the need to develop robust and effective infection control measures to prevent contamination of surfaces with pathogenic organisms. A critical component of such infection measures should include cost-effective continuous surveillance of environmental contamination with pathogenic bacterial species and adherence to established infection control measures to prevent the widespread dissemination of pathogens in hospital environments.

This study provides important information on the contamination of surfaces in sampled medical facilities with antibiotics resistant *A. baumannii* strains. However, the study is only limited to four medica facilities and the identification of *A. baumannii* strains based on cultural and biochemical characterristics could have underestimated the level of contamination, Therefore, further studies involving more medical facilities in the studied area employing molecular based identification will help in further quantifying the potential risk of *A. baumannii* contamination of surfaces in medical facilities in the studied district.

Contributions of author:

The study was solely conceptualised by the authors who conducted the study, generated the data, wrote and edited the manuscript for publication.

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Conflicts of interest

No conflict of interest is declared

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