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Multidrug-resistant Staphylococci Found on Book Surfaces in East London Libraries

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There is an increase in the presence of drug-resistant staphylococci outside of the nosocomial and health-care setting. Although the presence of staphylococci has been studied in several public spaces, nothing is known on the presence of staphylococci in public libraries. Book surfaces from public libraries in the East London area, United Kingdom were swabbed and cultured and identity of the isolates determined by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (MS). Seven different staphylococcal species were identified by MALDI-TOF-MS analysis. This short study provides evidence of the presence of multidrug-resistant staphylococci in public libraries in the East London area.

Keywords: Staphylococci, MALDI, non-hospital, antibiotic resistance, environment

Drug-resistant staphylococci have been a growing threat to the community and hospitals due to the misuse of antibiotics by humans, industrialization and lack of novel antimicrobials currently available. *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) are major contaminants of touch surfaces; they are pervasive in both clinical and non-clinical surfaces [1]. The transmission of staphylococci, including methicillin-resistant strains, occurs primarily via direct contact with these contaminated surfaces. The ability for staphylococci to survive for long periods of time on surfaces in public areas, where they frequently come into contact with people, can facilitate their easy transmission from person to person. The most well studied member of this genus is methicillin resistant *Staphylococcus aureus* (MRSA), a common hospital and environmental pathogen. It is now clear that there is a high number of *Staphylococcus aureus* and MRSA contamination in non-hospital environments [2]. There is a growing evidence to indicate an increase in the presence of drug-resistant staphylococci outside of the nosocomial and healthcare setting. There have been only a handful of studies investigating the incidence of multiple drug resistant staphylococci from non-healthcare environments including hotels [3], public restrooms [4, 5], public transport places [6–10], water parks [11], public telephones [12] and fitness centers [13–15]. However, the presence of drug resistant staphylococci has not been investigated in public library spaces. This study aims to investigate the presence of staphylococci in public libraries in the East London area to assess the level of antibiotic resistance in these bacteria. Seven different staphylococcal species were identified by Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) analysis. Out of the thirty-four staphylococci isolates, nine were resistant to multiple antibiotics including seven isolates which were oxacillin resistant. This short study provides evidence of the presence of multidrug-resistant staphylococci in

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public libraries in the East London area.

Dry sterile cotton swabs (Copan Diagnostics Inc, CA) were used to collect samples from selected book surfaces in public libraries in the East London area, United Kingdom. Approximately 4 cm^2 areas were sampled using dry cotton swab and stored in sterile swab collection tubes. A total of 100 swabs were collected. All specimens were transferred to the laboratory within 1–3 hours of the sample being taken. In the laboratory, the swabs were plated onto Nutrient Agar (Nutrient Agar, Oxoid, UK) medium before swabbing on to Mannitol Salt Agar (MSA, Oxoid, UK) selective medium to select for Gram-positive cocci. Plates were incubated for up to 48 h at 37℃ [16]. Bacterial colonies growing on MSA is then sub-cultured onto another MSA plate to ensure purity of the colonies.

Bacterial isolates were identified using MALDI-TOF-MS. Colonies (3–5) of overnight cultures were suspended in 300 µl distilled water. The suspension was mixed with 100% absolute ethanol and centrifuged for 1 min at 13,000 ×*g*. The pellets were re-suspended in 25 µl of 70% formic acid and then 25 µl pure acetonitrile was added. After mixing, solutions were centrifuged at 13,000 ×*g* for 2 min. One ml aliquots of the supernatant were spotted in duplicate onto MALDI ground steel targets, air-dried for 5 min at room temperature and each target spot was then overlaid with 1 µl a-cyano-4-hydroxycinnamic (HCCA) matrix solution. All isolates were purified and analyzed by MALDI-TOF-MS (Microflex LT, MALDI-TOF-MS, Bruker Daltonics, UK) in a positive linear mode (2000 to 20000 m/z range). The resulting spectra for each culture was analysed by MALDI-Biotyper 2.0 software (Bruker Daltonics, UK). The software evaluates each spectra compared to a reference spectra in the Bruker Taxonomy Database identifying the best match from database records. Results were expressed as scores (QI) from 0 to 3, as recommended by the manufacturer. Scores $QI \leq 1.7$ were not considered as reliable identification. A score of $QI \geq 1.7$ corresponded to 'genus' identification. Only scores higher than $QI \geq 2$ were considered a reliable identification of species.

Strains were then screened for resistance to antibiotics by agar disk diffusion on Iso Sensitest media (Iso Sensitest Agar, Oxoid, UK). Zones of inhibition were evaluated against twelve antibiotics (Oxoid, UK). The following antibiotic discs were used; chloramphenicol (30 µg), erythromycin (15 µg), fusidic acid (10 µg), oxacillin (1 µg), streptomycin (10 µg), tetracycline (10 µg), cefoxitin (30 µg), gentamicin (10 µg), vancomycin (5 µg), cefepime (30 µg), amoxicillin (10 µg) and mupirocin (20 µg). The minimum inhibitory concentrations (MICs) to oxacillin were additionally evaluated using "M.I.C. evaluators", antimicrobial gradient strips designed for accurate Minimum Inhibitory Concentration (MIC) values (Oxoid Ltd., UK). The categories susceptible, intermediate resistant or resistant were assigned on the basis of the CLSI antimicrobial susceptibility testing standards.

To determine the presence of the *mecA* gene, PCR amplification of the gene was performed. Fresh overnight cultures were centrifuged at 2 min at 4,000 ×*g* before decanting the supernatant. To extract DNA, bacterial pellets were then resuspended in 20 µl of sterile H_2O and subjected to warming at 100℃ for 10 min before cooling down at room temperature. The presence of the *mecA* gene was determined using primers described previously [5]. PCR thermal cycling conditions were 2 min at 94℃, 35 cycles for 30 s at 94℃, 30 s for 59℃ and 1 min for 72℃. The 2 log DNA ladder I (New England Biolab, UK) was used as molecular size markers.

94 out of the 113 isolates (83.7%) were identified using MALDI-TOF-MS (Table 1). The remaining 19 isolates (16.3%) failed to give a reliable identification. The rates of MALDI-TOF identification at the species level with a score of $QI \geq 2$ were 89.4% (84/94) and at genus level with a score of $1.7 \leq QI \leq 2$ were 10.6% (10/94). In this study, a large number of staphylococci were recovered from our samples. Overall, we identified 34 staphylococcal isolates belonging to 7 species. This included *Staphylococcus warneri* ($n = 6$), *Staphylococcus haemolyticus* ($n = 11$), *Staphylococcus hominis* ($n = 1$), *Staphylococcus*

Table 1. Summary of Family and Genera of bacteria identified by MALDI-TOF-MS.

Family	Genus	No of isolates
Staphylococcaceae	Staphylococcus	34
Dermabacteraceae	Brachybacterium	1
Micrococcaceae	Micrococcus	53
Micrococcaceae	Kocuria	3
Corynebacteriaceae	Corynebacterium	2
Intrasporangiaceae	Kytococcus	1
Total		94

saprophyticus (*n* = 2), *Staphylococcus capitis* (*n* = 3), *Staphylococcus cohnii* (*n* = 2) and *Staphylococcus epidermidis* (*n* = 9). We also identified several other bacteria such as, brachybacterium, micrococcus, corynebacterium, kocuria and kytococcus. Our findings highlight the complexity of the number of bacterial types that can be found on human contact surfaces on public library books but staphylococci were the most common.

Most of the staphylococci isolated in our study carried antibiotic determinants. All of the staphylococci isolates in this study were antibiotic resistant (Table 2). Nine of the isolates were resistant to five or more antibiotics out of which seven isolates were resistant to oxacillin including *Staphylococcus haemolyticus* (#7, #14) and *Staphylococcus epidermidis* (#23, #25, #26, #27) (Table 2). Methicillin resistance is commonly associated with the carriage of the *mecA* gene, which encodes penicillin binding protein, PBP2a. Our results also showed that seven

Table 2. Resistance profiles and molecular characterization of antibiotic resistant staphylococci isolated from book surfaces in public libraries in the East London area, United Kingdom.

#	Species	n	O	V	M	Cp	G	Fc	S	A	E	T	C	Cf	*MecA*
1	*S. haemolyticus*	1		R			R		R					R	
2	*S. warneri*	3		R					R						
3	*S. epidermidis*	1							R	R					
4	*S. warneri*	1		R				R	R		R	R			
5	*S. cohnii*	1		R				R	R		R				
6	*S. haemolyticus*	1							R						
7	*S. haemolyticus*	1	R	R				R	R		R				+
8	*S. saprophyticus*	1		R				R	R						
9	*S. captis*	2		R					R						
10	*S. epidermidis*	1		R	R	R			R	R					
11	*S. warneri*	1		R	R				R						
12	*S. warneri*	1		R					R	R					
13	*S. haemolyticus*	1		R	R			R	R						
14	*S. haemolyticus*	2	R	R					R	R				R	+
15	*S. haemolyticus*	2		R					R						
16	*S. haemolyticus*	2		R				R	R				R		
17	*S. saprophyticus*	1						R		R		R			
18	*S. hominis*	1		R											
19	*S. cohnii*	1						R							
20	*S. epidermidis*	1		R					R	R	R				
21	*S. haemolyticus*	1		R					R			R			
22	*S. captis*	1		R					R	R					
23	*S. epidermidis*	1	R	R				R	R	R	R	R			+
24	*S. epidermidis*	1			R				R						
25	*S. epidermidis*	1	R	R				R	R	R	R	R		R	+
26	*S. epidermidis*	1	R					R	R	R	R	R			+
27	*S. epidermidis*	1	R	R				R		R	R	R		R	+
28	*S. epidermidis*	1									R				

#, Assigned numerical ID
n, Similar isolates from each sampling location (i.e. library)
O: oxacillin; V: vancomycin; M: mupirocin; Cp: cefepime; G: gentamicin; Fc: fusidic acid; S: streptomycin; A: amoxicillin E: erythromycin; T: tetracycline; C: chloramphenicol; Cf: ciprofloxacin.

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of the oxacillin resistant staplylococci environmental isolates carried the *mecA* gene (Table 2). Our findings demonstrate that book covers from public libraries can indeed be a potential source of bacterial contamination. More importantly, our data provides evidence of the existence of multidrug resistant staphylococci in such public spaces. The presence of these bacteria in such environments provides further evidence that infection control measures, both in the hospitals and in public places, fails to limit the spread of multidrug resistant staphylococci and. This work represents an advance in biomedical science because this work has major implications in the issues of public health and safety outside of the healthcare setting hence emphasizing the importance of good hygiene in these environments.

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Conflict of Interest

Authors declare no conflict of interest.

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