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Original article

Antibiotic and Disinfectant Susceptibility Patterns of Airborne Bacteria Isolated from Restaurants in Nigeria

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Abstract

Antibiotics resistant airborne bacteria in restaurants have considerable effect on not only the life of food handlers, but also the quality and stability of food products. This study was carried out with the objectives of identifying the type of airborne bacteria associated with the restaurants and their susceptibility patterns to commonly used antibiotics and disinfectants. Using depositional sampling technique, air samples were collected from restaurant kitchens and dining rooms and cultured aerobically. Bacterial isolates were identified based on biochemical tests and selective/differential plating. Among the ten (10) bacterial species isolated and identified, Staphylococcus aureus 7 (19%), *Micrococcus* spp. 6 (17%), *Staphylococcus* spp. 5 (14%), and *Bacillus subtilis 4* (11%) were predominant. A total of fourteen antibiotics were used in this study: Amoxicillin + clavulanic acid (AU) (25), gentamycin (CN) (10µg), pefloxacin (PEF) (10µg), ofloxacin (OFX) (30µg), streptomycin (S) (30µg), chloramphenicol (CH) (30µg), co-trimoxazole. (SXT) (30µg), fluoroquinolone (SP) (10µg), ciprofloxacin (CPX) ((10µg)), amoxicillin (AM) (30µg), ampiclox (APX) (30µg), erythromycin (E) (19µg), ceftriaxone (CTR) (30µg) and cefuroxime (Z) (20µg). Antimicrobial susceptibility test results revealed that S. aureus had susceptibility of 6 (85.7%) each to ciprofloxacin and gentamycin but resistant to amoxicillin, ampiclox and cefuroxime while *Pseudomonas aeruginosa* had susceptibility of 3 (100%) each to amoxicillin + clavulanic acid and gentamycin but resistant to fluoroquinolone and co-trimoxazole. Susceptibility to Jik and Dettol was appreciable; they were bacteriostatic at 25-100% concentrations (Minimum Inhibition Concentrations (MIC) and bactericidal (Minimum Bactericidal Concentrations (MBC) at mostly 100% concentration. 'Mama Lemon' was bacteriostatic to only two isolates at 50 and 100% concentrations but not bactericidal. *Enterobacter* sp. was susceptible to neither the antibiotics nor the disinfectants. All the three disinfectants showed no efficacy at concentrations lower than 25%. The presence of potentially pathogenic bacteria which are not susceptible to antibiotics and disinfectants in the air of restaurants constitutes a serious health hazard not only to the restaurant workers and their customers, but also the general public.

Keywords: Indoor air contamination, Bioaerosols, Bacteria, Restaurants, Susceptibility pattern, Antimicrobials, Disinfectants, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration.

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INTRODUCTION

Monitoring airborne microorganisms in indoor air is one of the essential components of environmental monitoring (Kalwasińska *et al.,* 2012) and can be considered as a mirror of hygienic conditions of any place (Sabharwal and Sharma, 2015). Over the past several years the problem of indoor air pollution has received attention, with many studies on chemical and physical pollutants; however, less attention has been paid to pollutants of biological origin (Macher, 2017). In recent years, however, both scientific and public interests in indoor air pollutants have increased (Rajasekar and Balasubramanian, 2011). This is because poor indoor air quality has been shown to cause several health hazards (Laumbach and Kipen, 2005), and airborne microbiota, research has shown, greatly affect indoor air quality; for example, a large variety of nonspecific symptoms that occur in the residents of a building also called the sick building syndrome (SBS) (Joshi, 2008) is frequently linked to elevated levels to which airborne microorganisms occur in indoor air in typical enclosed spaces (Laumbach and Kipen, 2005; Teeuw *et al.*, 1994; Fischer and Dott, 2003).

Unlike viruses, bacteria and fungi grow, often to an alarming extent, on moist building materials. Inside buildings, levels of airborne bacteria and fungi change frequently as a result of human activity such as operation of mechanical air handling systems. In fact, building conditions such as moisture that allow excessive growth of bacteria or fungi can lead to occupants developing a number of medical symptoms (Menetrez *et al.,* 2007). Bacterial cells and spores transferred with airborne particles may pose source of contamination of processing surfaces and indirectly raw materials or final products (Faille *et al.,* 2014). Studies also indicate that air harbours an omnipresent bacterial community although the bacteria are low in abundance compared with, for example, bacteria in seawater and soil (Fahlgren *et al.,* 2010). Hernando *et al.* (2011) have reported the prevalence of airborne oxacillin-resistant *Staphylococcus aureus* from culturable air samples of urban residences. Presence of such antibiotic-resistant bacteria in air may cause serious health hazard to the people living in the area (Kabir *et al.,* 2016).

Antimicrobial agents are crucial in reducing the burden of infectious diseases worldwide (Antibiotic Expert Group, 2006). However, the development and spread of resistant strains of microorganisms, which is a major threat to global public (Finch, 2004; Kumari *et al.,* 2007), has continued to diminish the efficacy of many antimicrobials (Mandal *et al.,* 2009). This resistance to antibiotics and other antimicrobials, including the major last-resort drugs, poses alarming threat to public health (Daniel, 2004). One of the more disturbing recent trends in infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing infections (Diriba, *et al.,* 2016; Livermore, 2007; Moellering, 1998; Rhem and Weber, 2007). Although microbial resistance is not peculiar to poor countries alone, as it also poses a huge public health threat to the developed countries, the impact on poor countries has been proven to be pronounced (WHO, 2001; Hart

and Kariuki, 1998; Gallant, 2007). WHO (2012) as well as Mahmoud and Hanan (2012) reported that many factors play in the emergence of resistance which include but not limited to misuse of antimicrobial in the animal industry, poor utilization of antimicrobial agents, the transmission of resistant bacteria from patient to patient and from healthcare workers to patients and otherwise, a lack of guidelines for appropriate and judicious use of antimicrobial agents and lack of easy-to-use auditing tools for restriction.

Antiseptics and disinfectants particularly play an important role in infection control and the prevention of transmission of disease-causing microorganisms (Kumiko *et al.*, 2010). The mode of action of disinfecting agents is thought to be linked to the destruction of proteins, lipids or nucleic acids in the cells or its cytoplasmic membrane. Microorganisms' sensitivity to chemical agents may, however, vary from organism to another (Olowe *et al.*, 2004; Cheesbrough, 2005). The significance of the introduction of comprehensive disinfection on the reduction of healthcare-associated infections has been described (Makris *et al.*, 2000). However, a significant proportion of pathogens is not only resistant to disinfectants, but can also grow in them. Both growth and concentration of the colony forming units of bacteria at sites of application of disinfectants and antiseptics have been reported in the literature (Gajadhar *et al.*, 2003), but information about the linkage of resistance profiles to disinfectants and antimicrobial agents against a few bacterial species is available so far. Therefore, existing data seem insufficient for the empirical application of disinfectants (Clinical and Laboratory Standards Institute, 2009). The present study was carried out with the aim of isolating, identifying and determining antimicrobial profiles of airborne bacteria in restaurants.

MATERIALS and METHODS

Study Area/Location

The study was carried out in Kebbi State in the north-western part of Nigeria. Kebbi State is situated between latitudes 10° 8′ N – 13° 15′ N and longitudes 3° 30′ E – 6° 02′ E, with a population of 3,238,628 in 2006. The State occupies an area of approximately 36,229 square kilometres (Jirgi et al., 2016). The study was carried out in Birnin Kebbi, Jega and Aliero Local Government Areas of the State. Birnin Kebbi is the capital of the state, whereas Aliero and Jega are the commercial nerve centres of the state and were selected based on their strategic importance.

Samples Collection and Handling

A total of 19 restaurants were randomly selected for the study (Nickolas, 2007). Sampling for bacteriological analysis was carried out using open plate technique where Petri dishes containing Trypticase Soy Agar where exposed to the air for 15min at the height of approximately one meter. The Petri dishes were transported to the laboratory and incubated aerobically for 24 hours at 35°C - 37°C (Yassin and Almouqatea, 2010). A total of 684 samples were collected.

Isolation and Identification of Bacteria

To obtain pure bacterial isolates, a colony from mixed culture was picked using a sterile wire loop and placed on a fresh nutrient agar medium. After streaking, the Petri dish was incubated for 24 hours at 37ºC. All isolates from this pure culture were maintained in an agar slant for further analyses. All airborne bacterial isolates were identified according to their physical (colonial) characteristics (shape, colour, odour, pigmentation) and biochemical tests such as Gram's staining, Coagulase, Catalase, Indole, Urea, Citrate, Bacterial Spore stain, Motility test, Voges Proskauer test, Methyl red test and Oxidase test (Cheesbrough, 2003; Cheesbrough, 2006; Manga and Oyeleke, 2008). Additional selective/differential plating was employed to further identify the isolates:

Presumptive *Staphylococcus aureus* isolates were inoculated on mannitol salt agar and incubated at 37 ºC for 48 hours. Characteristic yellow colonies indicated *S. aureus* (Cheesbrough, 2003; Cheesbrough, 2006).

Presumptive *Pseudomonas aeruginosa* isolates were inoculated on Cetrimide selective agar at 37 ºC for 24 hours. Blue-green and yellow-green colonies indicated *P. aeruginosa* (Laine *et al*., 2009).

Presumptive *Streptococcus* spp were incubated on blood agar at 37 ºC for 24 hours. Light-yellow colonies indicated *Streptococcus pyogenes* (Cheesbrough, 2003; Cheesbrough, 2006; Manga and Oyeleke, 2008).

Antimicrobial Susceptibility Testing of Bacterial Isolates

Standardization of Test Organisms

A sterile loop was used to pick a loopful of freshly (24 hours) grown bacterial culture of the test organism. This was then transferred and suspended in a tube of sterile normal saline (for this purpose 8.5g NaCl, was dissolved in one litre of distilled water). The tube was compared with the turbidity standard and the density of the organism was adjusted to that of the standard by adding more bacteria or more sterile saline (Vandepitte *et al.*, 2003).

Antibiotic Susceptibility Testing of Bacterial Isolates

Antibiotics susceptibility of isolated bacteria was determined by the disk diffusion method (Kirby-Bauer), using Mueller-Hinton medium (Clinical and Laboratory Standards Institute, 2009).

Assessment of Disinfectants Activity against Bacterial Isolates

Preparation of the Disinfectants Concentrations

Different concentrations of disinfectants were prepared using 2-fold dilution technique (Okore et al., 2014; WHO, 2003). The local disinfectants used in this study were Dettol, JIk and 'Mama Lemon.'

Table 1. Disinfectants Used in the Study and Their Compositions

SN	Disinfectant	Ingredients
1	Dettol	Alcohol denat, aqua, PEG/PPG-17/16 copolymers, Acrylate/c10-30 Akyl Acrylate cross polymer, Tetrahydroxyl propyl Ethylenediamine, parfum, and limonene
2	Jik	Sodium hyphochlorite 3.5% m/v.
3	'Mama Lemon antibacterial'	Water, sodium benzole, EDTA-2NA, Kathon CG, SLES-2EO, P-77 surfactant, Na_2SO_4, fragrance

Impregnation of the Discs

The sterile 6mm diameter filter paper discs were impregnated with 0.1 mL each of the dilutions of the disinfectants using sterile pipettes (Okore *et al.*, 2014).

Determination of Bacterial Isolates Susceptibility to Disinfectants

A sterile cotton swab was dipped into a tube containing the standardized inoculum and rotated properly to allow maximum contact. The swab was firmly rotated against the inside of the tube above the liquid level in other to remove excess suspension. The swab was then streaked over the surface of sterile Mueller Hinton agar plate three times while rotating the plate through an angle of 60 after each application. The swab was also streaked around the edge of the agar surface. After the agar surface has absorbed moisture for about 5 minutes at room temperature, filter paper discs impregnated with the various concentrations of the disinfectants were placed at equidistance. Disc with no disinfectant was used as control. The diameters of the zones of inhibition were measured using a transparent milliliter ruler and the values obtained were compared with those of the interpretive chart for standardization adopted from Johnson and Case (1995). Diameter zone of inhibition of 10mm or less indicated test organism being resistant to test product, diameter zone of inhibition of 11 mm to 15mm indicated test organism being intermediate resistance to test product while diameter zone of inhibition of 16 mm or more indicated test organism being susceptible to test product.

Determination of Minimum Inhibitory Concentration (MIC)

Using broth dilution method, the disinfectants, which produced zones of inhibitions against the test bacterial isolates in the agar diffusion test, were further tested to determine their MIC values. Various concentrations of the disinfectants were prepared (50, 25, 12.5 and 6.25%.v/v). One millilitre of each disinfectant was introduced into tubes containing equal volume (1 mL) of standardized test organism. Each of the concentrations of the disinfectant was used in each case. A tube containing only the disinfectant and broth without bacteria was used as positive control while a tube containing only nutrient broth and test bacteria without disinfectant was used as negative control. The tubes were incubated for 24 hours at 37^{o}C and examined visually for presence of growth (turbidity) by comparing them with the control tubes. This was repeated for all the disinfectants. The lowest concentration of

disinfectant needed to prevent the growth of a given organism *in–vitro* is termed as its MIC (Nester *et al.,* 2009).

Determination of Minimum Bactericidal Concentration (MBC)

MBC was determined by assaying for live organisms in the tubes from the MIC tests, which showed no visible growth (Nester *et al.,* 2009). A loopful of inoculum from the MIC tubes that showed no visible turbidity was streaked onto fresh nutrient agar plates without the disinfectants incorporated into them. The plates were incubated at 37 ºC for 24 hours after which they were observed for growth. The lowest concentration of the disinfectants that produced no growth on the agar surface after 24 hours of incubation was regarded as its MBC against the test organism.

Statistical Analysis

The statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) version 20 to calculate mean diameter zone of inhibition of both antibiotics and disinfectants against every isolate.

RESULTS

Ten (10) bacterial species were identified. Seven species were found to be S. aureus, six Micrococcus spp., five Staphylococcus spp., four Bacillus subtilis, Streptococcus pyogenes, three species were found each of Pseudomonas aeruginosa and Corynebacterium sp., two species each of Proteus spp and Acinetobacter sp., and one species of Enterobacter sp. Table 2 shows the colonial, morphological and biochemical characteristics of the isolates. Results for the antibiotic susceptibility test of the isolates (Table 3) revealed that S. aureus had susceptibility of 6 (85.7%) each to ciprofloxacin and gentamycin, but resistant to amoxicillin, ampiclox and cefuroxime. P. aeruginosa had 3 (100%) each to amoxicillin + clavulanic acid and gentamycin but resistant to ampiclox, sparfloxacin and co-trimoxazole. Eenterobacter sp. was resistant to all the antibiotics tested. In the overall, 7 (88%), 30 (83%), 28 (78%) and 32 (61%) isolates were sensitive to augumentin, gentamycin, ciprofloxacin and co-trimoxazole respectively, whereas 1 (4%) and 5 (13%) were sensitive to ampiclox and amoxicillin respectively. All the isolates were resistant to sparfloxacin. Disinfectants susceptibility test revealed that susceptibility to Jik and Dettol was appreciable at 100% and 50% concentrations. They were bacteriostatic at 25-100% concentrations (MIC) and bactericidal (MBC) at mostly 100% concentration. However, Enterobacter sp. was not susceptible to any of the disinfectants. ‘Mama Lemon’ was less effective and was bacteriostatic to only two isolates at 50 and 100% concentrations but not bactericidal. All the three disinfectants showed no efficacy at concentrations lower than 25%. The results are presented in Tables 4 and 5.

Table 2. Colonial, Morphological and Biochemical Characteristics of Bacterial Isolates

S/N	COLONIAL MORPHOLOGY	MICROSCOPIC MORPHOLOGY	BIOCHEMICAL CHARACTERISTICS											No. of Isolates (%)	ORGANISM IDENTIFIED
			Grm	Sp	H2S	Ca	Co	Ox	Mr	VP	Ind	Cit	Ur	Mo		
	Circular, convex yellow	Cocci in bunches	+	-	-	+	+	NA	-	+	NA	+	+	-	7 (19.44)	*S. aureus*
	Small, round, entire, yellow	Cooci in pairs and tetrads	+	-	-	+	NA	NA	-	+	NA	-	+	-	6 (16.67)	*Micrococcus* spp.
	Pinpoint, circular entire, whitish	Cocci in chains	+	-	-	-	NA	NA	-	-	NA	+	-	-	3 (8.33)	*Streptococcus pyogenes*
	White raised colony	Cocci in bunches	+	-	+	+	-	NA	-	+	NA	-	+	-	5 (13.89)	*Staphylococcus* spp
	Creamish white, dry undulate irregular	Rod-shape	+	+	-	+	NA	NA	-	+	-	+	-	+	4 (11.11)	*Bacillus subtilis*
	Small, greyish, granular, translucent	Rod, forming V-shape	+	-	-	+/-	NA	NA	-	-	NA	-	+	-	3 (8.33)	*Corynebacterium* sp.
	Tan shiny, small entire, convex	Short rod, almost cocci	-	-	-	NA	NA	-	-	+	-	+	-	+	1 (2.78)	*Enterobacter* sp.
	Creamish white, circular smooth	Rod-shaped	-	-	+	-	NA	NA	-	+	-	+	+	+	2 (5.56)	*Proteus* spp.
	Non-pigmented, mucoid, round opaque	Cocco bacilli or diplococci	-	-	-	NA	NA	-	-	-	-	+/-	-	-	2 (5.56)	*Acinetobacter* sp.
	Circular, entire convex raised with green-blue pigmentation	Rod	-	-	-	NA	NA	+	-	-	-	+	-	+	3 (8.33)	*Pseudomonas aeruginosa*

Key: NA= not applicable; - = Negative, + = Positive, Gram's stain (Grm), Spore stain (Sp), Catalase (Ca), Oxidase test (Ox), Methyl red test (Mr), Voges proskauer test (VP), Indole (Ind), Citrate (Cit), Urea (Ur), Coagulase (Co), Motility (mo), Hydrogen sulphide (H2S)

Table 3. Bacterial Isolates Susceptibility to Antibiotics (values in brackets are percentages)

ISOLATES	No. of Isolates	ANTIBIOTICS													
		AM (30µg)	APX (30µg)	AU (25µg)	CH (30µg)	CPX (10µg)	CN (10µg)	E (19µg)	PEF (10µg)	OFX (30µg)	CTR (30µg)	SP (10µg)	SXT (30µg)	S (30µg)	CXM (20µg)
S. aureus	7 (19.4)	0 (0.0)	0 (0.0)	NT	NT	6 (85.7)	6 (86.7)	2 (28.6)	5 (714)	NT	2 (28.6)	NT	4 (57.1)	2 (28.6)	0 (0.0)
Micrococcus spp.	6 (16.7)	2 (33.3)	1 (16.7)	NT	NT	4 (66.7)	4 (66.7)	4 (66.7)	3 (50.0)	NT	3 (50.0)	NT	4 (66.7)	2 (33.2)	0 (0.0)
Streptococcus pyogenes	3 (8.3)	0 (0.0)	0 (0.0)	NT	NT	3 (100)	3 (100)	1 (33.3)	1 (33.3)	NT	1 (33.3)	NT	3 (100)	1 (33.3)	1 (33.3)
Staphylococcus spp.	5 (13.9)	1 (20.0)	0 (0.0)	NT	NT	4 (80.0)	5 (100)	2 (40.0)	2 (40.0)	NT	2 (40.0)	NT	1 (20.0)	3 (60.0)	0 (0.0)
Bacillus subtilis	4 (11.1)	2 (25.0)	0 (0.0)	NT	NT	3 (75.0)	3 (75.0)	2 (50.0)	2 (50.0)	NT	2 (50.0)	NT	3 (75.0)	2 (50.0)	2 (50.0)
Corynebacterium sp.	3 (8.9)	0 (0.0)	0 (0.0)	NT	NT	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)	NT	2 (66.7)	NT	3 (100)	2 (66.7)	0 (0.0)
Enterobacter sp.	1 (2.8)	0 (0.0)	NT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NT	0 (0.0)	0 (0.0)	NT	0 (0.0)	0 (0.0)	0 (0.0)	NT
Proteus spp.	2 (5.6)	0 (0.0)	NT	2 (100)	2 (100)	2 (100)	2 (100)	NT	2 (100)	2 (100)	NT	0 (0.0)	2 (100)	2 (100)	NT
Acinetobacter sp.	2 (5.6)	0 (0.0)	NT	2 (100)	0 (0.0)	2 (100)	2 (100)	NT	2 (100)	0 (0.0)	NT	0 (0.0)	2 (100)	2 (100)	NT
Pseudomonas aeruginosa	3 (8.3)	0 (0.0)	NT	3 (100)	1 (33.3)	2 (66.7)	3 (100)	NT	3 (100)	1 (33.3)	NT	0 (0.0)	0 (0.0)	1 (33.3)	NT

Key: Amoxicillin + clavulanic acid (AU), Gentamycin (CN), Pefloxacin (PEF), ofloxacin (OFX), streptomycin (S), chloramphenicol (CH), Co-trimoxazole (SXT), Sparfloxacin (SP), Ciprofloxacin (CPX), Amoxicillin (AM), Ampiclox (APX), Erythromycin (E), Ceftriaxone (CTR) and Cefuroxime (CXM). NT= not tested (antibiotics are absent in selected disc for Gram +ve or Gram -ve). 0.0 = Isolates are susceptible and n = Number of tested isolates.

Table 4. Susceptibility of Bacterial Isolates to Disinfectants (values in brackets are percentages)

Isolated bacteria	**Number of isolates**	**Disinfectants at Varying Concentrations (%)**											
		Jik				**Dettol**				**Mama Lemon**			
		100	**50**	**25**	**12.55**	**100**	**50**	**25**	**12.55**	**100**	**50**	**25**	**12.55**
S. aureus	7 (19.4)	6 (85.7)	1 (14.3)	0 (0.0)	0 (0.0)	6 (85.7)	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Micrococcus spp.	6 (16.7)	6 (100)	2 (33.3)	1 (16.7)	0 (0.0)	6 (100)	5 (83.3)	0 (0.0)	0 (0.0)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Streptococcus pyogenes	3 (8.3)	3 (100)	1 (38.3)	0 (0.0)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Staphylococcus spp.	5 (13.9)	5 (100)	3 (60.0)	1 (20.0)	0 (0.0)	5 (100)	3 (60.0)	2 (40.0)	0 (0.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)
Bacillus subtilis	4 (11.1)	3 (75.0)	1 (25.0)	1 (25.0)	0 (0.0)	4 (100)	2 (50.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)
Corynebacterium sp.	3 (8.9)	3 (100)	2 (66.7)	0 (0.0)	0 (0.0)	3 (100)	3 (100)	1 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter sp.	1 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Proteus spp.	2 (5.6)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Acinetobacter sp.	2 (5.6)	1 (50.0)	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pseudomonas aeruginosa	3 (8.3)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 5. MIC and MBC of Disinfectants against Bacterial Isolates

Organism	Isolate No.	Disinfectants					
		MIC (% Concentration)			MBC (% Concentration)		
		Jik	Dettol	Mama lemon	Jik	Dettol	Mama lemon
S. aureus	BI36	+	100	+	+	+	+
	B11	100	100	+	+	+	+
	BI5	100	50	+	+	100	+
	BI13	+	+	+	+	+	+
	BI20	50	100	+	100	+	+
	BI24	100	50	+	+	100	+
	BI32	100	50	+	+	100	+
Micrococcus spp.	BI3	100	50	100	+	100	+
	BI11	50	25		+	+	+
	BI16	100	100	+	+	+	+
	BI19	+	100	+	+	+	+
	BI28	25	25	+	100	100	+
	BI34	+	50	+	+	100	+
Streptococcus pyogenes	BI14	100	100	+	+	+	+
	B126	25	100	+	50	+	+
	BI30	50	100	+	100	+	+
Bacillus subtilis	B!4	+	100	+	+	+	+
	BI9	100	50	+	+	+	+
	BI15	25	100	+	100	+	+
	BI22	100	100	+	+	+	+
Corynebacterium spp.	BI18	100	50	+	+	100	+
	BI12	50	50	+	+	+	+
	BI35	50	25	+	+	100	+
Enterobacter sp.	BI17	+	+	+	+	+	+
Proteus spp.	BI29	100	100	+	+	+	+
	BI31	100	+	+	+	+	+
Acinetobacter spp.	BI2	+	+	+	+	+	+
	BI25	50	100	+	+	100	+
	BI7	100	+	+	+	100	+
Psedoemonas aeruginosa	BI21	-	100	+	+	+	+
	BI33	100	100	+	+	+	+
Staphylococcus spp.	BI6	100	25	+	+	100	+
	BI10	100	100	+	+	100	+
	BI18	25	50	+	100	+	+
	BI23	50	25	50	100	100	100
	BI27	50	50	+	100	100	+

Key: + = Presence of growth

DISCUSSION

A total of 10 bacterial species were identified from the indoor air of kitchens and dining rooms of the nineteen restaurants studied. The most predominant species were *S. aureus* 7 (19%), *Micrococcus* spp. 6 (17%), *Staphylococcus* spp. 5 (14%) and *Bacillus subtilis 4* (11%). The presence of cocci in the air of the restaurants can be linked to not only overcrowding but poor ventilation (Awad, 2007), and Gram-positive bacilli presence can be attributable to a number of outdoor sources, such as soil emissions, water, dust, air, faeces, vegetation, wounds and abscesses (Aydogdu *et al.,* 2010). The bacterial species identified in this study may further be verified by additional molecular methods or the API or Microgen Systems.

Different articles have similarly reported a wide number of microbial species in indoor environments. Safdar *et al.,* (2015) reported that predominant indoor air bacterial species from thirty (30) sampling sites were *Staphylococcus* spp. (37% in kitchens and 35.4% in living rooms), *Micrococcus* spp. (28.3% in kitchens and 29.8% in living rooms) and *Bacillus* spp. (11.8% in kitchens and 14.2% in living rooms) along with *Serratia* spp. and some unidentified Gram negative and positive rods and cocci in a few sites. *Micrococcus* spp. was reported by Pastuszka *et al.* (2000) to be present in all houses studied in Upper Silesia, Poland, with *Staphylococcus* epidermidis being present in 76% of houses studied (Pastuszka *et al.,* 2000). In another study, Gorny and Dutkiewicz (2002) recorded the presence of numerous bacterial species including *Aeromonas*, *Bacillus*, *Kocuria*, *Micrococcus*, *Nocardia*, *Pseudomonas* and *S.* in residential indoor environment. Joshi and Srivastava (2013) reported the presence of *Brevibacillus brevis*, *Arthrobacter* and *Bacillus cereus* (bacterial species) in indoor air.

In the present study, results revealed varying degrees of efficacy of antibiotics and disinfectants screened on the test organisms. Antibiotic susceptibility test results revealed that *S. aureus* was susceptible to 6 (85.7%) ciprofloxacin and gentamicin, but resistant to amoxicillin, ampiclox and cefuroxime each while *P. aeruginosa* had 3 (100%) each to Amoxicillin + clavulanic acid and gentamicin but resistant to ampiclox, sparfloxacin and co-trimoxazole. *S. aureus* high sensitivity to ciprofloxacin as found in this study, is closely comparable with the results obtained by Alamin *et al.* (2013) and Teshome *et al.* (2016) where *S. aureus* isolates were found to be 89.5%, and 77.8% sensitive to ciprofloxacin respectively (Alamin *et al.,* 2013; Teshome *et al.,* 2016). Similar to the results obtained in the present study, NNIS (2004) as well as Ogunnusi and Adeyinka (2016) have identified gentamicin-sensitive *P. aeruginosa* from clinical and environmental samples.

Enterobacter sp.*,* as the present study has demonstrated, was resistant to all the antibiotics screened. The *Enterobacter* which is a member of *Enterobacteriaceae,* is a Gram negative, rod shaped and non-spore forming bacteria, live as facultative anaerobic and have been reported as significant opportunistic and multi resistant bacterial pathogens of humans (Mezzatesta *et al*., 2012). Species of *E. aerogenes,* for example, are naturally resistant to ampicillin, amoxicillin-clavulanate, cefazolin and

cefuroxime (De Gheldre *et al.,* 1997). It generally exhibits high resistance to broad-spectrum antibiotics, which is as the result of enzymatic responses, mutations in the antibiotic target, and modifications in envelope permeability, including porin alteration and induction of drug efflux as documented by Chikere *et al.* (2008).

Overall, 87.5%, 83.3%, 77.8% and 61.1% of the isolates were susceptible to Amoxicillin + clavulanic acid, gentamycin, ciprofloxacin and co-trimoxazole respectively. Sparfloxacin, ampiclox and amoxicillin had 0%, 3.6% and 11.1% respectively. High level of susceptibility to gentamicin seen in the present study was also recorded by Chikere *et al*., (2008), who demonstrated that 93.3% of the isolates were sensitive to gentamicin. Kabir *et al*. (2016) documented 100% level of *S. aureus* susceptibility to gentamicin. The low sensitivity of the isolates generally to streptomycin, ampiclox, cefuroxime and amoxicillin could be linked to common use of these antibiotics. As Chollet *et al.,* (2004) indicated that single drug treatment can lead to cross-resistance to other unrelated antibiotics. This agrees with findings of Kabir *et al.,* (2016), Kumurya *et al.* (2010) and, Tagoe *et al*. (2011) who in their different works, linked antibiotic resistance to misuse of antibiotics in chemotherapy. Similarly, spread of resistant bacterial strains is facilitated by inter species gene transmission not only poor sanitation and hygiene in communities and hospitals, but also the increasing frequency of global, travel, trade, and disease transmission (Ramanan *et al.,* 2013).

El-Mahmood and Doughari (2009), Rutala *et* al., (2000), Thomas *et al.,* (2012), Otokunefor and Usoh (2009), Ghotaslou and Bahrami (2012) and Iruoha *et al.,* (2011) have documented the antimicrobial properties of Dettol, Jik and other disinfectants. In the present study susceptibility to Jik and Dettol was appreciable at 100% and 50% concentrations. They were bacteriostatic at 25-100% concentrations (MIC) and bactericidal at mostly 100% concentration. However, *Enterobacter* sp. was not susceptible to any of the disinfectants. 'Mama Lemon' was less effective and bacteriostatic to only two isolates at 50 and 100% concentrations but not bactericidal. All the three disinfectants showed no efficacy at concentrations lower than 25%. Antimicrobial activity of disinfectants is affected by a number of factors such as the type, concentration and volume of alcohol used, the contact time, the test method (*in-vitro* and *in-vivo),* target organism and matrix (CDC, 2004). High antimicrobial activity observed in Jik and Dettol can be attributable to the presence of sodium hypochlorite and denatured alcohol respectively as active components. Alcohols are known to exert disinfectant activity in bacteria by causing protein denaturation, disruption of tissue membranes and dissolution of several lipids (Kar, 2016). The findings of the present study are in agreement with the findings of Okere *et al.,* (2014), who reported that although disinfectants had remarkable zones of inhibition against bacteria and fungi with Dettol showing broad spectrum activity, test microorganisms differ in their susceptibilities to the disinfectants. Findings of Awodele *et al.* (2007) indicated that Jik at 100% concentration, inhibited the growth of *P. aeruginosa, B. subtilis* and *C. albicans* to a level of 17, 15 and 18 mm, respectively; at 50%

concentration, its inhibitory activity on *P. aeruginosa* and *B. subtilis* reduced to a level of 15 and 4 mm, respectively, and there was no inhibition on *C. Albicans*. Similarly, the MIC and MBC of disinfectants from the present study, to some extent, agrees with the results obtained by Oke *et al.*, (2013) who reported that Hygel and Dettol were at 100% concentration bacteriostatic (MIC) and none was bactericidal to *S. aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia.*

Conclusion

The indoor air quality of the restaurants studied was found to be poor, contaminated with potentially pathogenic bacteria which are resistant to both antibiotics and disinfectants. The presence of such organisms in indoor air of restaurants constitutes serious hazard to not only restaurant workers, food preparation surfaces, raw and ready-to-eat food, but also customers and the general public.

Competing Interest

The authors do not have any competing interest.

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