SAUDI BIOLOGICAL SOCIETY

ORIGINAL ARTICLE

Antibiogram profiling and pathogenic status of *Aeromonas* species recovered from Chicken

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Abstract   Poultry meat and its products are widely consumed by humans globally, however, *Aeromonas* infections in poultry have been reported in different parts of the world with devastating effects. This study was carried out to assess the antibiogram and biofilm forming potential of *Aeromonas* isolated from chicken fecal samples. *Aeromonas* isolates were screened for antibiotic susceptibility using antibiotics disk and biofilm producing potentials on abiotic surfaces. Nineteen isolates recovered from chicken feces were 100% sensitive to ciprofloxacin, gentamicin and the tetracyclines. About 53% of *Aeromonas* isolates were resistant to erythromycin and 47% resistant to streptomycin. Eight isolates (42.1%) were found to be moderate producers of biofilm, 31.6% (6/19) were weak producers of biofilm, 10.5% (2/19) were non biofilm producers while 15.8% (3/19) were strong producers. The present investigation shows a prevalence of potentially pathogenic *Aeromonas* strains in chicken feces, suggesting potential group at risk for *Aeromonas* infection which could be dissemination to other animals or humans with close contact and the wider community.

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1. Introduction

Poultry meat and its products are among the most widely consumed food all over the world. Chicken meat is delicious, nutritious and a good source of protein characterized by good flavor and easily digested. *Aeromonas* infections in poultry have been reported in different parts of the world with

devastating effects (Dashe et al., 2013). A higher occurrence of *Aeromonas* from chicken source (Smita and Brahmabhatt, 2011) suggests that chicken could be a potential host for the spread of *Aeromonas* infection and present a possible threat to public health. Considering the high frequency of *Aeromonas* in poultry stool samples (Jindal et al., 1993), poultry carcasses and poultry plant processing water (Barnhart et al., 1989; Zanella et al., 2012), there is need to investigate the presence of *Aeromonas* in chicken samples.

Aeromonas species has the ability of colonizing several ecological niches. *Aeromonas* intestinal colonization is as a result of several virulence factors. Virulence in *Aeromonas* is multifactorial and not yet understood. Microbial colonization of mucosal surfaces is a complex process which results in infection, however, for most microbial infections it is thought to

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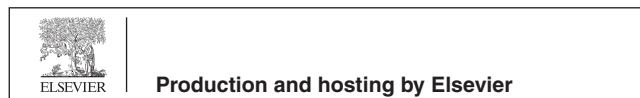
involve biofilm formation. A biofilm is an accretion of organisms entrenched in a polysaccharide matrix of their own making and adhering to a surface. Bacteria in biofilms are free-floating planktonic cells and show more resistance to antimicrobial agents and host defenses. They may also express more virulent phenotypes as a result of gene activation through bacterial communication ("quorum sensing") or gene transfer (Greenberg, 1999; Costerton et al., 1999; Kirov et al., 2002). An important component in biofilm formation is the ability to move over and colonize surfaces after the initial attachment.

Aeromonas species have been described as an emerging food borne pathogen involved in human gastroenteritis ranging from mild diarrheal to cholera-like illness (Vila et al., 2003; Igbinosa et al., 2012). Aeromonas has also been implicated in meningitis, cellulites, otitis, endocarditis, osteomyelitis, peritonitis, bacteremia, and septicemia, among others diseases (Albert et al., 2000; Zanella et al., 2012). Aeromonas spp. have emerged as important human pathogens associated with food borne disease outbreaks (Fukushema et al., 2007; Awaad et al., 2011; Dashe et al., 2013). Biochemically, Aeromonas are Gram negative rods, oxidase and catalase positive, facultative anaerobe, motile, glucose fermenting. The ubiquitous nature of Aeromonas in aquatic, clinical and environmental sources has made it possible for forms of life such as human and domestic animals to have close contact with and become infected with Aeromonas species. Aeromonas have been implicated in water and food borne disease outbreak in different parts of the world especially in developing countries where hygiene and access to quality water supply is a challenge (Odeyemi and Ahmad, 2013).

The use of antibiotics has been vital in the treatment of infectious diseases caused by bacteria which has contributed to the rise in average life expectancy in the Twentieth century. However, bacteria that cause disease have become resistant to antimicrobial chemotherapy and are an increasing public health challenge (Sharma et al., 2010). The antibiotic susceptibility of an isolate is usually required for effective clinical control.

Microbial resistance to antibiotics is partially as a result of bacterial dynamism in adapting to its environment as well as increasing use, and misuse, of existing antibiotics in agriculture, human and veterinary medicine. Antimicrobial resistance among enteric pathogens is a part of major problem in developing countries where there is a high occurrence of gastroenteric illnesses and many antibiotics fall routinely into inadequate use. Antibiotic resistance is mostly pertinent in pathogenic Aeromonas species due to the frequent occurrence of multiple antibiotic resistances besides the classical resistance to beta-lactamic antibiotics (Kampfer et al., 1999; Vila et al., 2002). Also, these bacteria could possess integron (Igbinosa et al., 2013) which enables them to receive and transfer antibiotic resistance genes, giving rise to the risk from resistant bacterial infections (Marchandin et al., 2003; Zanella et al., 2012). There is a need for periodic surveillance of drug resistance of these organisms in different geographical areas and different sources for appropriate guidance for choice of antimicrobial agent for empiric therapy.

2. Materials and methods

2.1. Isolation procedure

The survey area of the study was Fort Cox agricultural farm in the Eastern Cape province of South Africa. Chicken fecal samples were collected at random in the months of September and December 2010 from the poultry segment of the farm and transported to the laboratory in cold chain. About 10 g of samples was inoculated in buffered peptone water (pH 7.2) for enrichment and incubated at 36 °C for 18–24 h. Enriched culture media were spread onto GSP agar plates for Aeromonas isolation. After 24 h incubation, phenotypic yellow colonies were picked and purified (Igbinosa et al., 2013). Pure colonies were transferred unto nutrient agar plates and slants. Isolates were identified based on biochemical characteristics using API 20NE profiling kit. The strips were then read, and final identification was made using API lab plus software (bioMerieux, Marcy l'Etoile, France).

2.2. Antibiotic phenotyping of isolates

Antibiotic susceptibilities of isolates were carried out using the following antibiotics: amoxycillin (30 μg), ampicillin–sulbactam (20 μg), aztreonam (30 μg), cefotaxime (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), neomycin (30 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), oxytetracycline (30 μg), penicillin G (10 μg), streptomycin (10 μg), tetracycline (10 μg), tobramycin (10 μg), trimethoprim–sulfamethoxazole (25 μg). Antibiotics were selected on the basis of antibiotics used as food additive in agriculture and those used for the treatment of Aeromonas associated infections. Pure isolates were grown on nutrient agar plates for 18 h afterward 4–6 colonies were suspended in normal physiological saline and adjusted to turbidity of 0.5-M McFarland standard. Subsequently, the isolate suspension was spread onto Muller Hinton agar (biolab) plates. Plates were allowed to dry and impregnated with the appropriate antibiotic disks. Plates were incubated at 36 °C for 24 h after which zones of inhibition were measured and recorded (Igbinosa et al., 2013). The inhibition and zone margins were selected as the areas showing no visible growth. The sizes of the zones were interpreted using published standards of the Clinical Laboratory Standard Institute Guidelines (CLSI, 2006) and the isolates reported as susceptible, intermediate or resistant against the antimicrobial agents tested.

2.3. Biofilm formation assay

Aeromonas isolates were grown for 18 h in trypticase soy broth at 36 °C and centrifuged for 2 min at 12,000 rpm. Cell pellets were washed and re-suspended in phosphate-buffered saline (pH 7.2) turbidity equivalent of 0.5 McFarland standard (Basson et al., 2007). Wells of sterile 96-well U-bottomed polystyrene microtiter plates was inoculated with 180 μl trypticase soy broth and 20 μl of standardized cell suspensions in order to determine bacteria adherence to abiotic material (Jacobs and Chenia, 2011; Igbinosa et al., 2013). Aeromonas hydrophila ATCC 7966 was used as positive control while wells containing only broth were used as negative control. Microtitre plates were incubated at 36 °C for 24 h. The absorbance reading of each well was obtained at 570 nm using an automated microtiter-plate reader (Synergy mx Biotek^R USA). Assays were done in triplicate and the results averaged (Jacobs and Chenia, 2011; Igbinosa et al., 2013). Biofilm formation was classified as non-adherent, weakly, moderately or

strongly-adherent. The cut-off OD (ODc) for the microtiter plate test was defined as three standard deviations above the mean OD of the negative control. Isolates were classified as follows: $OD \leq OD_C$ = non-adherent, $OD_C < OD \leq (2 \times OD_C)$ = weakly adherent; $(2 \times OD_C) < OD \leq (4 \times OD_C)$ = moderately adherent and $(4 \times OD_C) < OD$ = strongly adherent (Jacobs and Chenia, 2011; Igbinosa et al., 2013).

3. Results and discussion

3.1. Occurrence of Aeromonas species

Biochemical identification of isolates was carried out using API 20NE kit. Based on biochemical identification of isolates, six *Aeromonas* species isolates were recovered from fecal samples collected in September 2010 while thirteen *Aeromonas* isolates were recovered from fecal samples collected in December 2010 resulting in a total of nineteen (19) isolates. Although, other microorganisms might have been present in the samples, however, *Aeromonas* was the interest microorganism of study. The isolation media used differentially and selectively narrowed the possibility of other microorganisms. *Aeromonas* have the capability of adapting to different ecological niche (Mateos et al., 1993; Arora et al., 2006) and possess astonishing properties which permit their survival and ability to survive and flourish in diverse condition (Agarwal, 1997; Arora et al., 2006), thereby allowing their cosmopolitan occurrence in nature. *Aeromonas* recognition as an emerging food borne pathogen is on the increase.

3.2. Antibiotic phenotyping of isolates

Most of the *Aeromonas* isolates were resistant to erythromycin (macrolides) but were sensitive to tetracycline, chloramphenicol, nitrofurantoin, quinolone, fluoroquinolones and aminoglycosides as shown in Table 1. The tetracyclines showed absolute sensitivity against all *Aeromonas* isolates. A similar observation of *Aeromonas* susceptibility to tetracycline has been reported in different geographical regions (Zanella et al., 2012; Awan et al., 2009; Mahmoud and Tanios, 2008). Although tetracycline has been used as growth stimulant in poultry feed for many years, the low resistance *Aeromonas* observed against tetracycline indicate minimal repercussion of tetracycline as antibiotics of choice in poultry farming. The aminoglycosides (gentamicin and tobramycin) showed brilliant activity against *Aeromonas* isolates with gentamicin showing absolute activity (Table 1). In a study carried out by Awan et al. (2009), *Aeromonas* isolated from food samples including fresh and frozen chicken demonstrated absolute sensitivity to gentamicin. This also corroborates with the report of (Dallal et al., 2012) who found excellent gentamicin activity against *Aeromonas* isolated from minced meat and chicken samples. The cephalosporins (cefotaxime) showed very high potency against the isolates compared to (cephalothin) which showed approximately average activity, which signify that *Aeromonas* species have variable susceptibility to cephalosporins. *Aeromonas* resistance to first generation cephalosporins, is expected given that motile *Aeromonas* demonstrate beta-lactamase activity and frequently the presence of metallo-beta-lactamases of expanded effect (Morita et al., 1994).

Trimethoprim-sulfamethoxazole complex showed appreciable susceptibility against *Aeromonas* isolates and resistance rate of 36.8%. This observation is similar to reports of (Zanella et al., 2012) and corroborates the finding of Ghenghesh et al. (2013), in which all *Aeromonas* isolates from chicken samples showed absolute sensitivity to Trimethoprim-sulfamethoxazole. Although, some literature argue the effectiveness of sulfamethoxazole–trimethoprim (SXT) complex against *Aeromonas* as a result of reports of other authors showing *Aeromonas* resistance (von Graevenitz and Altwegg, 1991). However, a study carried out on sulfamethoxazole alone against *Aeromonas* isolates showed poor activity but as a complex SXT, its efficacy against the isolates improved significantly (Awan et al., 2009), which is in conformity with the result obtained in the present study.

The penicillins (amoxycillin and penicillin) were absolutely inactive against all isolates while a slight variability of Ampicillin–sulbactam was insignificant. Several researchers have documented *Aeromonas* resistance to penicillins (Kaskhedikar and Chhabra, 2009; Zanella et al., 2012; Ghenghesh et al., 2013). In general, Most *Aeromonas* isolates are intrinsic or chromosomally mediated resistance against ampicillin (Rall et al., 1998). The resistance is as a result of at least four β-lactamases (von Graevenitz and Altwegg, 1991; Awan et al., 2009). Apart of the antimicrobial agent that showed absolute activity, the following antibiotics showed excellent activity (> 70% strains were sensitive) against all the isolates tested. These include aztreonam, cefotaxime, chloramphenicol, nalidixic acid, nitrofurantoin, and tobramycin. A similar observation of *Aeromonas* sensitivity to cefotaxime, chloramphenicol, nitrofurantoin and tobramycin has been documented (Awan et al., 2009) while *Aeromonas* sensitivity to aztreonam, nalidixic acid among others has been reported (Zanella et al., 2012).

Aeromonas has been reported to show excellent sensitivity against chloramphenicol nitrofurantoin and tetracycline (von Graevenitz and Altwegg, 1991; Pasquale et al., 1994; Vivekanandhan et al., 2002). A similar observation has been documented (Awan et al., 2009) which is in accordance with the result obtained in the present study.

3.3. Biofilm formation assay

Biofilm formation is considered a vital virulence factor, aiding bacterial colonization by cell adhesion to epithelial cells and intestinal villi, reducing bacterial sensitivity to antimicrobial agents, and the reducing recognition of the bacteria by the immunologic system (Davey and O'Toole, 2000; Zanella et al., 2012). A quantitative assessment of biofilm producing potential of *Aeromonas* isolates showed significant variation among the isolates. Eight isolates (42.1%) were found to be moderate producers of biofilm while 6 (31.6%) of the isolates were weak producers of biofilm. In general, 2 (10.5%) isolates were non producers of biofilm whereas 3 (15.8%) were strong producers as shown in Fig. 1. The existence of biofilm forming *Aeromonas* from poultry and poultry workers has been documented (Zanella et al., 2012), which corroborates the result of this study. *Aeromonas* biofilm forming ability was also reported by Kirov et al. (2002), and was considered a potential virulence factor.

In conclusion, the present investigations show a high prevalence of potentially pathogenic *Aeromonas* strains in chicken

Table 1 Patterns of *Antibiotic phenotype* of *Aeromonas* isolates from chicken feces.

Antimicrobial agents	Susceptible		Intermediate		Resistance	
	No.	%	No.	%	No.	%
Amoxycillin (30 µg)	0	0	0	0	19	100
Ampicillin–sulbactam (20 µg)	2	10.5	0	0	17	89.5
Aztreonam (30 µg)	16	84.2	0	0	3	15.8
Cefotaxime (30 µg)	16	84.2	2	10.5	1	5.3
Cephalothin (30 µg)	11	57.9	3	15.8	5	26.3
Chloramphenicol (30 µg)	14	73.7	1	5.3	4	21.1
Ciprofloxacin (5 µg)	19	100	0	0	0	0
Erythromycin (15 µg)	6	31.6	3	15.8	10	52.6
Gentamicin (10 µg)	19	100	0	0	0	0
Kanamycin (30 µg)	12	63.2	0	0	7	36.8
Nalidixic acid (30 µg)	15	78.9	1	5.3	3	15.8
Neomycin (30 µg)	12	63.2	4	21.1	3	15.8
Nitrofurantoin (300 µg)	15	78.9	0	0	4	20.1
Norfloxacin (10 µg)	13	68.4	1	5.3	5	26.3
Oxytetracycline (30 µg)	19	100	0	0	0	0
Penicillin G (10 µg)	0	0	0	0	19	100
Streptomycin (10 µg)	8	42.1	2	10.5	9	47.3
Tetracycline (10 µg)	19	100	0	0	0	0
Tobramycin (10 µg)	17	89.5	0	0	2	10.5
Trimethoprim-sulfamethoxazole (25 µg)	10	52.6	2	10.5	7	36.8
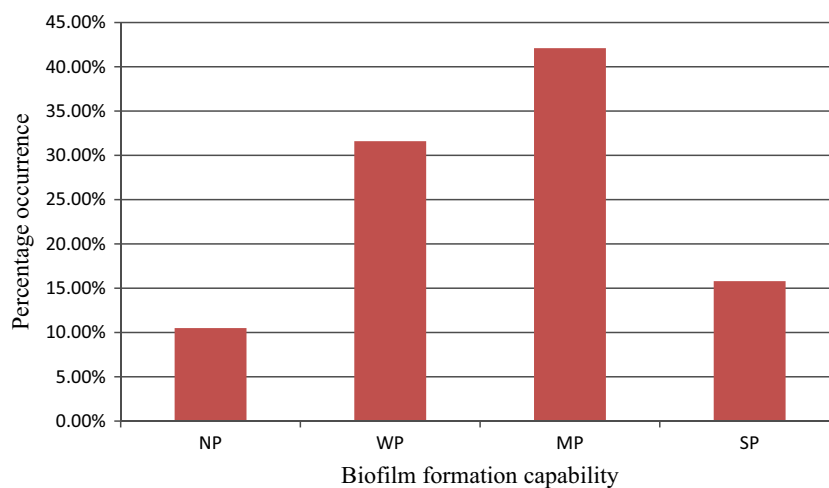
Legend: NP- non-producer, WP-weak producer, MP-moderate producer, SP-strong producer

Figure 1   Biofilm producing potential of *Aeromonas* isolates isolated from chicken feces.

feces. This suggests a potential group at risk for *Aeromonas* gastroenteric diseases and the dissemination of *Aeromonas* to other animals or humans with close contact and the wider community. Periodical screening of poultry birds across different geographical location is essential.

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