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Full Length Research Paper

Public health implication of Listeria species and other bacteria isolates of abattoir effluent in Lagos, Nigeria

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Abstract

Untreated abattoir effluent constitutes a reservoir for the spread of intestinal pathogens and *Listeria species* (though rarely considered), is one of such organisms. This study was therefore conducted to determine the status of these bacteria and others in abattoir effluent, in Lagos, Nigeria. Thirty samples of abattoir effluent were collected over a period of 6 weeks at the government central abattoir in Lagos, Nigeria. Each sample was serially diluted and pour-plated on Nutrient Agar, MacConkey Agar and Listeria Selective Agar. Mesophilic aerobic counts were enumerated. Isolated bacterial colonies were identified by standard methods and antimicrobial susceptibility test conducted using the disk diffusion technique. Heavy loads of *Listeria* species, *Escherichia coli*, *Klebsiella,* sp., *Enterococcus faecalis,* and *Pseudomonas, aeruginosa*, were isolated from all the samples. The antibiotic susceptibility pattern of these bacterial organisms revealed marked resistance to most of the antimicrobial agents tested. With the exception of *Pseudomonas*, there was no statistically significant difference between the antimicrobial resistance rate of *Listeria* and other bacteria isolates (P >0.05). The public health significance of these findings, particularly the abattoir effluent bacteria potential capability of transferring disease and antibiotic resistance to man, as well as the challenges posed to disease treatment was highlighted.

Keywords: Abattoir effluent, *listeria* species, bacteria isolates, public health, antimicrobial resistance.

INTRODUCTION

Abattoir effluents are waste water derived from animal slaughtering activities in abattoirs, consisting mainly of intestinal contents, blood and water. Abattoir effluent like other types of discharged sewage, eventually enter natural bodies of water like ground water, streams, rivers, lakes and oceans as a result of natural drainage pattern and sequence (Madigan *et al.*,1997; Pelczar *et al.*, 2002). These water bodies are used by human beings for drinking, household, industrial, agricultural (irrigation), swimming and other recreational purposes.

Drinking water and recreational water have been implicated in the transmission of pathogens, and it was opined that the source of contamination could be either sewage or infected animals (Muniesa *et al.*, 2006; Sehgal *et al.*, 2008).

A number of bacteria species, including coliforms and *Listeria* can be present in the Intestines of some humans and animals, including birds without causing infection (Ramaswany *et al.*, 2007).

The Genus *Listeria* consists, mainly, of 8 species, namely *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria welshimeri*, *Listeria grayi, Listeria marthii* and *Listeria rocourtiae* (Liu 2006; den Bakker *et al.*, 2010). Out of these eight species of *Listeria*, only *Listeria monocytogenes* (pathogenic to human and animals) and *Listeria ivanovii* (pathogenic to animals) are regarded as pathogens, while all other species are generally regarded as non-pathogenic (Law and Donachie 1997; Liu 2006). However, there have been, of recent, reported cases of human infection with *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua* and *Listeria welshimeri* (Rocout *et al.,* 1986; Andre and Genicot 1987; Allenberger 2002; Perrin *et al.,* 2003). *Listeria monocytogenes* is an intracellular, food – borne and zoonotic pathogen. It is the aetiological agent of the

disease, listeriosis (Portnoy *et al*., 2002; Chen *et al*., 2007; Rebagliati *et al.,* 2009). Listeriosis is a regularly reported disease in Europe and North America but only a few sporadic cases have been reported in Africa and other developing countries where the food industry is not very developed (Ennaji *et al*., 2008).

There are invasive and non – invasive forms of infection with *Listeria monocytogenes* (Françiosa *et al*., 2001; Vazquez-Boland *et al*., 2001). The non – invasive form which is characterized by gastroenteritis in the absence of more serious symptoms like septicemia, meningitis, abortion etc, following food borne infection with *Listeria monocytogenes,* has only recently been definitively determined by Dalton *et al*., 1997 (Dalton *et al*., 1997; Ramaswany *et al.* 2000, Françoisa *et al.,* 2001). It has been suggested that the occurrence of non-invasive listeriosis may be underestimated as *Listeria monocytogenes* is not among the pathogens routinely investigated in outbreaks of gastro - intestinal disease (Franciosa *et al*., 2001; Ramaswany *et al*., 2007).

The disposal of abattoir effluent which feeds natural bodies of water and the monitoring of the bacterial status of such effluent are of public health significance (Madigan *et al*., 1997; Black *et al*., 1998), especially in developing countries like Nigeria, where abattoir effluent are discharged untreated. Abattoir effluent, like other types of industrial sewage are supposed to undergo various stages of treatment to eliminate or remove bacterial content before being discharged into drainage to enter the natural bodies of water (Hug *et al*., 2005; Nestar *et al*., 1998).

Furthermore, the presence of various types of bacteria species in abattoir effluent makes it a conducive environment for the transmission of antimicrobial resistance amongst them (Mach and Grimes, 1982). Antimicrobial resistance has generally undergone near exponential increase in the past decades (Safdar and Armstrong, 2003). Prophylactic use of common broad spectrum antibiotics as well as empirical preemptive therapy in high risk settings, or indiscriminate usage, particularly in developing nations, has further accentuated this trend, especially in patients with underlying malignancy(Safdar and Armstrong, 2003; Bondarinzadeh, 2007).

Listeria organisms are generally known to be antibiotic susceptible in developed nations (Boisivon *et al*., 1990). The situation in Nigeria and other African countries is not well known, but bacteria generally, are known to be resistant to commonly used antibiotics like ampicillin, chloramphenicol, tetracycline, septrin etc (Akano *et al*., 2009).

Moreover, studies have shown that plasmids carrying antibiotic resistant genes can successfully transfer genetic codes from *Enterococcus faecalis* to *Listeria monocytogenes* (Poyart –Salmeron *et al.,* 1990). This observation has raised serious concerns regarding possible emergence of antibiotic resistance and the choice of optimal initial therapy for severe listeric infection especially in compromised individuals (Safdar and Armstrong, 2003).

There is the need to draw attention of governments, managers of abattoir operation and the public to the possible health consequences of the disposal of untreated abattoir effluent. This study was therefore carried out to identify the bacterial organisms of public health significance associated with abattoir effluent from Lagos, Nigeria. Furthermore, the study will determine the response of the organisms to antimicrobial agents, as a mean of contributing to the state of knowledge on the treatment of diseases associated with these organisms.

MATERIALS AND METHODS

Sample

Thirty samples of abattoir effluents were collected into sterile 100ml bottle aseptically. The effluent were collected from the drainage point immediately after the slaughter slab where the solid part (sludge) of the sewage was separated with the use of 'wire mesh' to allow the free flow of effluent. Thirty samples of 50ml each of effluent were collected, one sample per day, consecutively for 5 days per week, with the total collection spanning a total of 6 weeks. Each sample was transported to the laboratory and analyzed immediately.

The sampling was carried out at the government central 'Abattoir and Lairrage' in Lagos, Nigeria. At this abattoir about 1,250 heads of cattle are slaughtered daily for consumption in Lagos, Nigeria. This represents about 65% of the total heads of cattle (about 1,923) in all government approved abattoirs in Lagos State, Nigeria.

Isolation and Identification of Organisms

Serial decimal dilution (10^{-1}, 10^{-2}…10^{-8}) of each sample of well mixed effluent was prepared in a conical flask using sterile distilled water as diluents. A flask containing only sterile distilled water (no effluent) was included as control.

One milliliter of each dilution and the control were pour - plated with 20ml melted (and cooled to 45^0C). Nutrient agar (Biotec, U.K.), MacConkey agar (Biotec, U. K.) and Listeria Selective Agar (Oxoid, England) on separate petri dishes. The poured plates were allowed to set (solidify), inverted and incubated at 37^0C for 18-24 Hrs (Pelczar *et al*., 2002). Mesophilic aerobic counts were enumerated on Nutrient agar, coliform counts were determined on MacConkey agar and *Listeria* counts were determined on Listeria Selective Agar. Plates containing colonies ranging from 30-150 were selected for counting and recording. Both *Staphylococcus* and *Bacillus* (diptheroids) counts were not determined, although they

were infrequently isolated on MacConkey agar. Suspected colonies of Bacillus were tested for presence of spores after Gram-staining, while those of suspected *Staphylococcus* were subjected to catalase and coagulase tests after the Gram's staining result was confirmed. Typical representative colonies of isolates were sub-cultured on fresh MacConkey agar plates and black colonies of *Listeria* species on Listeria Agar were sub-cultured on Nutrient Agar for purity. Their presumptive identity were verified by microscopy, Gram staining and biochemical tests. The identification of the isolates was carried out in accordance with standard methods of identification of bacteria of medical importance (Cowan, 1993).

Antimicrobial Susceptibility Testing

The identified isolates were subjected to antimicrobial susceptibility test, using the disc diffusion technique as described by Bauer *et al* (1966). Antimicrobial mutidiscs (Antec Diagnostics, U.K.) and single discs containing the following antibiotics were applied: Amoxicillin (5ug); Tetracycline (25ug); Chloramphenicol (10ug); Streptomycin (25ug); Septrin (25ug); Gentamycin (10ug); Ofloxacin (10ug); Augmentin (30ug); Ciprofloxacin (10ug). The zones of inhibition observed were compared with those of reference organism, *Escherichia coli* NCTC, 10418 to determine susceptibility or resistance to antibiotics tested.

Antimicrobial Susceptibility Test Data Analysis

Analysis of Variance (ANOVA) test was used to detect differences in the susceptibility/resistance rate of the bacteria species isolates to antimicrobial agents. A difference at 5% level was considered to be statistically significant.

RESULTS

Five major bacteria were isolated, identified and enumerated in all the 30 samples of abattoir effluent examined in this study. They included *Listeria* sp., *Escherichia coli*, *Klebisella sp*, *Enterococus faecalis and Pseudomonas aeruginosa*. The mean coliform count on MacConkey agar was 2.8 x 10^6 cfu/ml while the aerobic mesophilic count on Nutrient agar was 4.1 x of 10^7 cfu/ml. The *Listeria* sp. count on Listeria Agar was 733cfu/ml. *Staphylococcus* species and diphteroids were also isolated less frequently during the study (Table 1).

Antimicrobial Susceptibility Pattern

All the bacterial isolates showed marked resistance to the antimicrobial agents tested, in varying degrees (Table 2). *Pseudomonas aeruginosa* isolates were the most resistant of all the bacterial species. They were resistant to all the antimicrobial agents with the exception of ofloxacin (Table 2). Generally, the bacterial isolates showed more susceptibility to the third generation antibiotics (i.e. ciprofloxacin, ofloxacin) than to the first generation group (i.e. chloramphenicol, tetracycline and septrin). With the exception of *Pseudomonas aeruginosa*, there was no statistically significant difference between the antimicrobial resistance rate of *Listeria species* and other bacteria isolates ($P > 0.05$).

DISCUSSION

The public health significance and concern over the sources and nature of water for consumption, food preparation, irrigation and recreation in any community is worldwide. This is due to the fact that water is known to be most potent vehicle of transmission of infectious diseases (Nester *et al.,* 1998; Muniesa *et al*., 2006). Abattoir effluents, when discharged, find their way into the natural drainage pattern and sequence. (Madigan *et al*., 1997; Pelczar *et al*., 2002) and could therefore be a source of contamination of drinking or recreational water (Muniesa *et al*., 2006).

In this study, abattoir effluent was found to contain several millions of *Enterococcus faecalis*, *Pseudomonas aeruginosa, Escherichia coli, Klebsiella sp*., *Listeria* species etc. The presence of these enteric bacteria and other bacterial species, apart from being potentially pathogenic or opportunistically pathogenic, also indicate the possible presence of pathogenic enteric organisms such as *Salmonella sp*., *Campylobacter jejuni* and *Listeria monocytogenes* (Black *et al.,* 1998).

The presence of *Listeria* sp. in abattoir effluent is particularly significant because the non-invasive form of listeriosis which is characterized only by gastroenteritis, in the absence septicemia; meningitis etc. has only been recently definitively determined by Dalton *et al*., 1997. Although the *Listeria* isolates were not identified to species level, nor their virulence determined within the scope of this study, their mere presence in abattoir effluent is of public health significance as many *Listeria* species apart from *Listeria monocytogenes*, which are hitherto regarded as non-pathogenic to human, such as *L. ivanovii, L. seeligeri, L. innocua L. welchimeri* have been incriminated in human infections (Rocourt *et al*., 1986; Andre and Genicot, 1987). Furthermore, it is

Table 1. Bacterial isolates and counts of abattoir effluents

Bacterial Type	Culture Media	Total Count (cfu/ml) for 30 samples	Mean Count (cfu/ml)	Isolate Identify
Coliforms	MacConkey Agar	$83.6x10^6$	$2.8x10^6$	• *Escherichia coli* • *Klebsiella sp.* • *Enterococcus faecalis* • *Pseudomonas aeruginosa* • *Staphylococcus spp.* • Diphtheroids
Aerobic Mesophillic Count (AMC)	Nutrient Agar	123.6×10^7	4.1×10^7	
Listeria count	Listeria Selective Agar	2.3×10^4	733	*Listeria* species

Table 2. Antibiotic susceptibility pattern of bacterial isolates of abattoir effluent; *Antibiotic Sensitivity Profile (expressed as percentage of total number of isolates tested)*

ISOLATES (NUMBER)	AMX	TET	CHL	STR	SEP	GEN	OFL	AUG	CIP	ERY
Escherichia coli (60)	21	80	25	100	55	0	100	75	100	ND
Klebsiella sp. (60)	19	95	40	100	65	55	100	55	100	ND
Pseudomonas aeruginosa (60)	0	0	0	20	0	0	100	0	30	ND
Enterococcus faecalis (60)	12	55	75	55	ND	65	ND	55	100	0
Listeria sp	14	64	92	78	72	96	100	23	96	79

TET = Tetracycline; CHL = Choramphenicol; STR = Streptomycin; SEP = Septrin OFL = Ofloxacin; AUG = Augmentin; CIP = Ciprofloxacin; AMX = Amoxicillin; GEN = Gentamycin; ND = Not determined

believed that the occurrence of non-invasive listeriosis is underestimated because L. *monocytogenes* is not among the pathogens routinely investigated in the outbreaks of gastro-intestinal diseases (Franciosa *et al.,* 2001; Ramaswany *et al*., 2007).

In the developed nations of the world, there are government agencies with relevant laws and standards guiding the treatment and disposal of abattoir effluents in order to protect the health of the people. These laws make the treatment of abattoir effluents before discharge mandatory for operators of the abattoir (FEPA, 1991). In Nigeria, there are similar agencies with relevant laws and standards guiding the treatment and discharge of effluent (FEPA, 1991; LASEPA, 1996).

However, while these laws and standard are enforced and adhered to in the developed nations like United States of America and United Kingdom, there is no such enforcement and adherence in Nigeria.

Abattoir effluents are discharged untreated into the drainage system, with the consequent health hazard to the populace.

A number of studies conducted outside Nigeria, have linked outbreak of some strains of pathogenic *Escherichia coli* and *Listeria monocytogenes* to animal source (Keen *et al*., 2006; Ramaswany *et al*., 2007). Studies conducted in Nigeria have not definitely linked the contamination of food by enteric organisms to animal source. However there are strong indications that such contaminations are from water sources (Olasupo *et al*., 2002b). These water sources, especially in rural areas could have been rivers, streams or groundwater into which discharged, untreated effluent has gained access to.

This study in addition to determining the identity and bacterial load (count) of abattoir effluent isolates, have also determined their antibiotic susceptibility pattern. Most of the isolates were found to be resistant to the commonly used antimicrobial agents like tetracycline, Chloramphenicol, septrin and amoxicillin in varying degrees. It is also significant to know that there is no statistically significant difference between the resistant rate of *Listeria* species and other bacteria isolates of abattoir effluent in this study ($P>0.05$). This is at variance with previous findings from developed countries where *Listeria species* are susceptible to most commonly used antibiotics(Boisivon *et al*,1990).It is however not surprising giving the rate of abuse of antibiotics which promotes the acquisition of resistance genes by bacteria in developing nations like Nigeria(Akano *et al*., 2009). This finding is also in consonance with findings in previous studies conducted where high resistance level was observed among food and clinical/human – bacterial isolates (Ebigwei and Olukoya, 1991; Olasupo *et al*., 2002a). This high resistance level among food bacterial

isolates have been partly attributed to possible transfer of resistance trait from indigenous microflora associated with the sources of the raw materials used in the preparation of these foods (Olasupo *et al*., 2002b). Since water is one of such materials used, contamination from abattoir effluent isolates is quite a possibility.

In this study as well as earlier studies conducted in Nigeria, there was no definite investigation to confirm the linkage of outbreaks of diseases and antibiotic resistance to abattoir effluent. There is therefore the need to conduct further studies to confirm this linkage, as a means of controlling the transfer and spread of infectious diseases and antibiotic resistance through abattoir effluent. There is also the need for more focus on *Listeria species* as a possible cause of human gastroenteritis.

In conclusion, this is the first known study on the bacterial status of abattoir effluent with particular reference to *Listeria* in Nigeria, and it has drawn attention to the importance of enforcing relevant laws on the treatment of abattoir effluent before discharge. This will not only prevent the transmission of pathogenic organisms to the public but also control the transfer and spread of antibiotic resistance through abattoir effluent, in view of the generally high resistance of the bacterial isolates of abattoir effluent to antimicrobial agents in this study. Bacterial resistance to antimicrobial agents has grave consequences and compounds the problem of disease treatment.

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