Original Research Article

ANTIBIOTIC SUSCEPTIBILITY OF BACTERIA ISOLATED FROM ABATTOIR EFFLUENT-IMPACTED TAGANGU RIVER, ALEIRO, KEBBI STATE, NORTH-WESTERN NIGERIA

ABSTRACT

This study aimed to evaluate the impact of abattoir effluent on microbiological quality of the receiving Tagangu River and the susceptibility of isolates to commonly-used antibiotics. The total heterotrophic population as well as *Escherichia coli* O157:H7 numbers in a total of 30 water samples collected over a period of three months at three strategic points of the river indicated that the river has been heavily polluted with the effluent discharges and did not meet any of the WHO guidelines for natural water sources fit for irrigation or other domestic purposes. In accordance with CLSI guidelines, four of the eight bacteria (*Enterobacter* sp., *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Citrobacter* sp.) isolated, demonstrated multiple antibiotic resistance (MAR) against at least three of septrin, chloramphenicol, amoxicillin, augmentin, gentamicin, tarivid and streptomycin. All the isolates (*Escherichia coli, Klebsiella pneumoniae*, *Enterobacter* sp., *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Citrobacter* sp., *Serretia marcescens* and *Aerobacter aerogenes*) showed either high or intermediate susceptibility to sparfloxacin, ciprofloxacin and pefloxacin. Indiscriminate discharge of abattoir effluent could impact on the microbiological quality and promote increased incidence of multiple antibiotic resistant bacteria in a receiving river.

Keywords: Abattoir, effluent, Tagangu River, Microbiological quality, antibiotic susceptibility Test.

1.0 INTRODUCTION

Abattoir waste disposal in many developing countries including Nigeria has been a major challenge for years [1]. In most cases, waste materials are disposed of without regard to sound environmental management practices, thus making them harmful to humans and other terrestrial and aquatic life [2]. Studies from Nigeria and Ghana show that many abattoirs in the respective countries either deposit waste materials in the immediate environs or dispose of them directly into water bodies, some of which serve as sources of water for the abattoirs [3].

The major known sources of water pollution are municipal, industrial and agricultural. The most polluting of them are sewage and industrial waste discharges into rivers. Industrial effluents mostly contained microbes, heavy metals, acids, hydrocarbons and atmospheric depositions [4].

In Nigeria, Meat processing activities are generally carried out in unsuitable buildings and by untrained personnel or butchers who are most of the time unaware of sanitary principles [5]. The major activities involved in the operations of an abattoir are: receiving and holding of livestock; slaughter and carcass dressing of animals; chilling of carcass products; carcass boning and packaging; freezing of finished carcass and cartooned product; rendering processes; drying of skins; treatment of wastes and transport of processed materials [5].

1.1 Abattoir Effluent as a Pollutant

In Nigeria, available reports cite gross contamination of most major river bodies across the nation by discharge of industrial effluents, sewage and agricultural wastes among others [6]. Abattoir activities may be another source of water pollution since human activities such as animal production and meat processing have been reported to impact negatively on soil and natural water composition leading to pollution of soil, natural water resources and the entire environment [7].

Yahaya *et al.,* [8], reported that animals which graze on contaminated plants and drink from polluted waters, as well as marine lives that breed in heavy metal polluted waters also accumulate such metals in their tissues and milk if lactating. When such animals are killed, these metals are released in the soil as natural sink but subsequently leached out into nearby streams or water bodies.

1.2 Impact of Untreated Abattoir Effluent

The continuous drive to increase meat production for the protein needs of the ever increasing world population has some problems attached [9]. In developing countries like Nigeria, water pollution from abattoir frequently arises from activities in meat production as a result of failure in adhering to good manufacturing practices and good hygienic practices [10].

Discharge of abattoir wastewater to surface waters affects the water quality. One of the environmental effects of discharging slaughterhouse wastewater causes de-oxygenation of rivers and the contamination of groundwater [11, 12, 13]. Moreover, discharge of high levels of biodegradable organics into receiving streams results in increased microbial activity associated with excessive nutrient loadings which requires greater amounts of oxygen than natural aeration processes. This decreases the available dissolved oxygen which negatively affects aquatic organisms [14].

A specific example of what happen is logging of contaminated water in the soil. In that situation, oxygen become less, available as an electron acceptor, promoting denitrifying bacteria to reduce available nitrate into gaseous nitrogen which enters the atmosphere with resultant negative effects [13]. Also, anaerobic archaea (methanogens) may produce excessive methane at a high rate than aerobic methane oxidizing bacteria (methanotrophs) could cope with, there for contributing to greenhouse effect and global warming [15]. Increasing in methane is of concern because it is five times more effective as a greenhouse gas than carbon dioxide (CO_2).

Wrongful discharge of blood and animal faeces into streams may cause oxygen-depletion as well as nutrient over enrichment of the receiving system which could cause increased rate of toxin accumulation [16]. Humans may also be affected through outbreak of water borne diseases and other respiratory and chest diseases [17].

1.3 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. After the revolution in the "golden era", when almost all groups of important antibiotics (Tetracycline, Cephalosporin, Aminoglycosides and Macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance [18]. Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health [19].

2.0 MATERIALS AND METHODS

2.1 Study Area

The study area was a section along the Tagangu seasonal River at old Kasuwa (Market) area located in Sarkin Fada 1 Ward Aleiro, Kebbi State, Nigeria. Kebbi State was created on 27th August in 1991 from the old Sokoto State. It is located in the North Western part of Nigeria between the latitude 11.6781^0N and longitude 4.0695^0 E. According to the 2011 National Population Census (NPC) estimate, the total population of Kebbi State is 3,802,500. Its capital city is Birnin Kebbi.

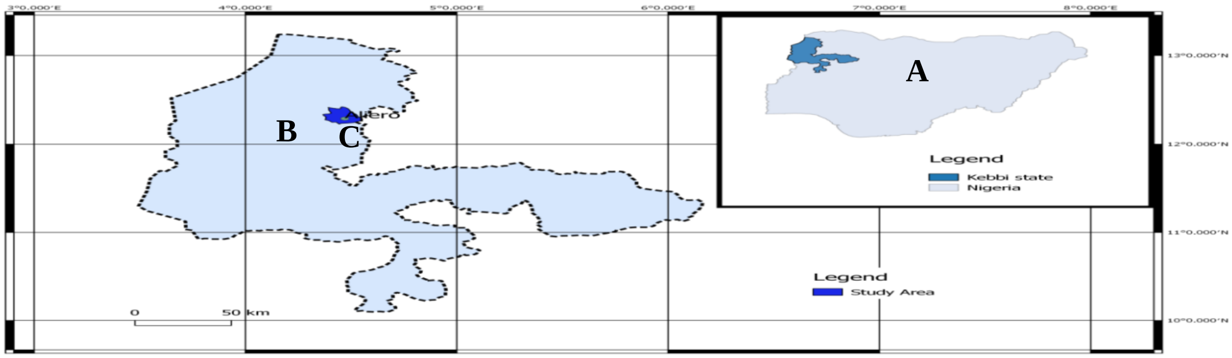

Fig. 1: Map of Nigeria (A) Kebbi State map (B) Aliero map (C)

2.2 Samples Collection and Preparation

A total of thirty (30) water samples were collected, ten (10) each from the three sections named as downstream, upstream and irrigation site denoted as A, B and C respectively, along the Tagangu seasonal River receiving the abattoir effluent. The water samples were collected as described by Cheesbrough, [19], the water samples were collected at the point's representative of the sampling sites (A, B and C) and transported to the laboratory in an ice jacket box and subsequently processed within 4 hours of sampling.

2.3 Media Preparation

All the media used under this study were prepared as described by [20].

2.4 Bacteriological Analyses

2.4.1 Isolation and Identification of Bacterial Isolates

The organisms were isolated and identified based on colonial morphology, cultural characteristics and biochemical tests as described by [20, 21, 22].

2.5 Antibiotic Susceptibility Test Profile of the Isolates

The antibiotic susceptibility testing (Agar disk diffusion method) of the isolated organisms was carried out in accordance with the standard approved by the Clinical and Laboratory Standards Institute (CLSI) [23].

2.6 Statistical Analyses of the Results

ANOVA system of analysis was carried out using SPSS computer application. The results were typed, analyzed and interpreted.

3.0 RESULTS AND DISCUSSION

3.1 Bacteriological Analyses

3.1.1 Total heterotrophic bacteria plate count

Table 1 represents the number of the heterotrophic bacterial count (cfu/ml). Sample A had the highest count of $1.64\pm1.94 \times 10^7$ cfu/ml, followed by sample C with the count of $1.62\pm1.69 \times 10^7$ (cfu/ml), while the least count of $1.57\pm1.64 \times 10^7$ (cfu/ml) was observed in sample B.

The total heterotrophic bacterial plate count recorded was highest in samples A ($1.64\pm1.94 \times 10^7$ cfu/ml) followed by samples C ($1.62\pm1.69 \times 10^7$cfu/ml), while the lowest number of $1.57\pm1.64 \times 10^7$ cfu/ml was observed in samples B. This is so because samples A were obtained from upstream, where the incoming substances including microbes do reside before getting to other portions of the river, it's also a point at which abattoir effluent directly find their way into the river body without treatment, and that must contained high level of contamination.

Samples B were also collected from downstream where the effluent has to travel far away to get to the site, while samples C were also obtained from a place called irrigation space; where the farmers use the water for growing crops, and therefore was expected to have a fair number of microbial count, but much physicochemical contaminations. This was in agreement with what

UNESCO [24] reported that agricultural run-off is another major water pollutant as it contains nitrogen compound and phosphorus from fertilizers, pesticides, salts, poultry wastes and washes down from abattoirs. Contaminants are usually of varied composition ranging from simple organic substances to complex organic compounds with varying degrees of toxicity.

Table 1: Total Heterotrophic Bacterial (THB) Plate Count

Samples	**Total heterotrophic bacterial count (cfu/ml)**
A	$1.64 \pm 1.94 \times 10^{7}$
B	$1.57 \pm 1.64 \times 10^{7}$
C	$1.62 \pm 1.69 \times 10^{7}$

Keys: cfu/100ml= Colony forming unit/100ml.

3.1.2 The frequency and percentage occurrence of identified organisms

Figure 2 represents the frequency and percentage occurrence of the identified bacteria from the water samples. *Escherichia coli* have the highest percentage occurrence of 56.7% while *Aerobacter aerogenes* has the least of 20%.

The frequency and percentage of isolates reported in this study indicates that *Escherichia coli* have the highest occurrence of 17 and a percentage of 56.7% while *Aerobacter aerogens* have the lowest occurrence of 6 and the percentage of 20%. This was contraindicated with the statement of International Reference Center for Community water supply and sanitation, which stipulated that, the level of coliforms which should be presence in any giving water body should be less than 10/100ml of a sample, and the number of *E. coli* should be less than 2.5/100ml of a sample.

The bacteria isolated from the River Tagangu were enterobacteriaceae. The presence of enteric bacteria like *Serratia marcescens*, *Salmonella species*, *Shigella species, Klebshialla species* and

Escherichia coli O157:H7 can be attributed to high level of faecal, municipal and abattoir waste contaminations which may constitute health hazard to the people drinking or using the water for domestic activities or both. The high incidence of Enterobacteriaceae recorded in this study could be due to the virulent factors present within these organisms which gives them the ability to be resistant to antibiotics.

The result of this study also agreed perfectly with the similar result carried out by Olayemi and Oyadege, [25], were as high as 45.3% incidence of Enterobacteriaceae among other organisms were recorded in Gombe state, Nigeria. Similarly *Escherichia coli* was also incriminated as the highest organism (36.6%) that was isolated from the gastrointestinal tract of fresh water fish as reported by Trust [26].

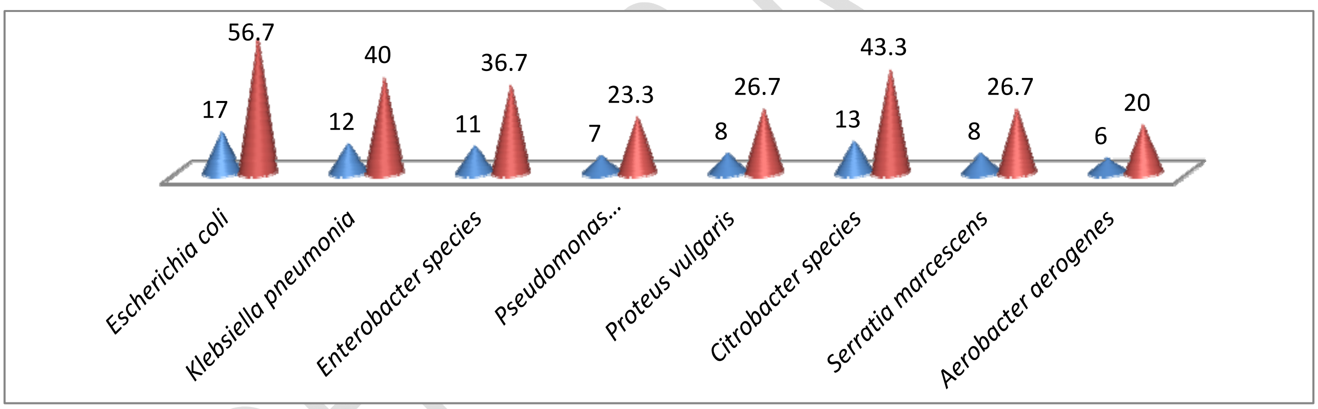

Figure 2: Frequency and percentage (%) occurrence of identified bacteria

3.2 Antibiotic Susceptibility Test Profile

Table 2: represents antibiotic susceptibility profile test of each of the identified organisms in each of the antibiotic disc tested. *Escherichia coli* indicate the highest zone of inhibition of 18.6±0.06mm with Sparfloxacin, Amoxicillin and Tarivid respectively, and the least of 16.6±0.04mm with Septrin. *Klebsiella pneumoniae* demonstrates the highest zone of inhibition of 19.3±0.07mm with Tarivid, and the least of 15±0.03mm with Gentamycin.

Enterobacter species points the highest zone of inhibition of 21±0.09mm with Augmentin, and the least of 12.3±0.01mm with Gentamycin. *Pseudomonas aeruginosa* indicates the highest zone of inhibition of 18.3±0.06mm with Ciprofloxacin, and the least of 13.3±0.01mm with Tarivid. *Proteus vulgaris* counts the highest zone of inhibition of 19.6±0.07mm with Amoxicillin, and the least of 14.3±0.02mm with Augmentin. *Citrobacter species* happens to have the highest zone of inhibition of 20.6±0.08mm with Tarivid, and the least of 15.6±0.03mm with Amoxicillin.

Serratia marcescens reveals the highest zone of inhibition of 17±0.05mm with Ciprofloxacin and Pefloxacin respectively, and the least of 12±0.01mm with Amoxicillin. *Aerobacter aerogenes* indicates the highest zone of inhibition of 19±0.07mm with Amoxicillin, and the least of 14±0.02mm with Augmentin and Septrin respectively.

The antibiotic susceptibility profile of all the identified bacteria tested, *Enterobacter species* revealed the highest zone of inhibition of 21±0.09mm with Augmentin, followed by *Citrobacter species* with the zone of inhibition of 20.6±0.08, while the least zone of inhibition of 12±0.01 was observed with *Serratia marcescens.* This finding is similar to the some previous investigations in other regions carried out in non-domestic environment [27, 28], the findings stated that *Serratia marcescens, Citrobacter and Enterobacter species* were investigated to have the highest resistant with most antibiotics in non-domestic environment in Portugal.

Table 2: Antibiotic susceptibility test profile of the identified organisms from the water samples

Antibiotics	Potency	*Escherichia coli*	*Klebsiella pneumonia*	*Enterobacter species*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Citrobacter species*	*Serratia marcescens*	*Aerobacter aerogenes*
	Zones of inhibition (mm) measured								
SXT	30µg	18.3±0.06	17.3±0.05	00.0±0.00	15.3±0.03	17±0.05	19.6±0.07	16.6±0.04	16±0.04
CH	30µg	17.3±0.05	17.3±0.05	00.0±0.00	15±0.03	18±0.06	00.0±0.00	16.3±0.04	14.6±0.02
SP	10µg	18.6±0.06	19±0.07	19.3±0.07	17.3±0.05	18±0.06	17.3±0.05	16.6±0.04	16±0.04
CPX	10µg	17.3±0.05	18±0.06	19±0.07	18.3±0.06	18.6±0.06	17.3±0.05	17±0.05	16.3±0.04
AM	30µg	18.6±0.06	18±0.06	16.6±0.04	17±0.05	19.6±0.07	15.6±0.03	12±0.01	19±0.07
AU	30µg	00.0±0.00	16.3±0.04	21±0.09	00.0±0.00	14.3±0.02	00.0±0.00	13.5±0.1	14±0.02
CN	10µg	00.0±0.00	15±0.03	12.3±0.01	00.0±0.00	15±0.03	00.0±0.00	14±0.02	15±0.03
PEF	30µg	17.3±0.05	17.3±0.05	15.6±0.03	16.3±0.04	17.3±0.05	17.6±0.05	17±0.05	15.6±0.03
OFX	10µg	18.6±0.06	19.3±0.07	00.0±0.00	13.3±0.01	19±0.07	20.6±0.08	16.3±0.04	17.3±0.05
S	30µg	16.6±0.04	00.0±0.00	13.3±0.01	00.0±0.00	15.3±0.03	16.3±0.04	14±0.2	14±0.02

Keys: SXT= Septrin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciproflaxacin, AM= Amoxicillin, AU= Augmentin, CN= Gentamycin, PEF= Pefloxacin, OFX= Tarivid, S= Streptomycin.

4.0 CONCLUSION

The high level of enteric pathogens demonstrated in Tagangu seasonal River located at Shiyar Fada 1, Aleiro Local Government, Kebbi State Nigeria, which always receives a tremendous amount of Aleiro abattoir effluent, and their multiple resistance to commonly used antibiotics, further confirmed the dangers associated with discharging municipal waste, organic waste and untreated wastewater to the river, which have a fatal impact on the river and its users. Therefore, it has been concluded that the water from the river is microbiologically unhygienic and unsafe for domestic (washing of clothes, animal products and feeding of animal) and agricultural purposes (growing of crops) without bacteriological treatment.

CONFLICT OF INTEREST

There was no conflict of interest exist

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