Health Risk Assesment of Air Emitting from Different Abattoirs in Akure Metropolis of Ondo State, Nigeria.

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ABSTRACT

The burden of diseases can be greatly reduced by reducing air pollution which is one of the greatest risks to health in our environments. The study assessed the different types of micro-organisms present in a major and minor abattoir in Akure metropolis of Ondo state, Nigeria and their sensitivity to Antibiotics. Pre-tested questionnaires were used to obtain socio-demographic information, how the environment of the abattoir is sanitized and diseases likely to be contacted from the workers working in the abattoirs. Different levels of occurrence of isolates were observed in the major and minor abattoirs and the total count was done with the total bacteria counts ranging from lowest to highest. The total count for *Escherichia coli* range from 3.6×10 cfu/ml to 6.5 ×10 cfu/ml, while the total bacteria count ranged from 1.34×10 cfu/ml to 2.55×10 cfu/ml, also total coliform ranged from 2.8×10 cfu/ml to 4.1×10 cfu/ml at the major abattoir. The total count for *Escherichia coli* range from 5.7×10 cfu/ml to 7.2×10 cfu/ml, while the total bacteria count range from 5.2×10 cfu/ml to 1.56×10 cfu/ml, also total coliform ranged from 3.4×10 cfu/ml to 4.3×10 cfu/ml at the minor abattoir. During gram staining test it was observed that three out of the eight organisms namely, *Micrococcus luteus, bacillus spp, Staphylococcus aureus,* were gram positive, while *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Enterobacter aerogenes* and *Aeromonas spp*were gram negative. Antibiotic sensitivity was carried out on each of the isolates. Ciprofloxacin antibiotic had inhibitory effect on both grampositive bacteria with the highest effect on *Staphylococcus aureus* at 24mm and gram negative bacteria with highest result on *Escherichia coli* at 21mm. Therefore, there is presence of pathogenic micro-organisms especially *Escherichia coli* in the air environment of the abattoirs. Conclusively, there is need for routine environment sanitation of these slaughter houses because inhalation of air from these abattoirs can constitute a great environmental menace to the workers and residents around the area where they are situated, resulting in health complications to those with existing health challenges.

Key Word: Abattoir, Microbial analysis, Air sampling, Air pollution, Antibiotic, Sensitivity pattern.

INTRODUCTION

Air is a mixture of gases. The common name ‘air’ is given to the atmospheric gases used in breathing and photosynthesis. By volume, dry air contains 78.9% nitrogen, 20.95% oxygen, 0.93% argon, 0.039% Carbon dioxide and small amount of water vapor, on average around 1% at sea level and 0.4% over the entire

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atmosphere.

The concentration of water vapor varies significantly from around 10ppmv in the coldest portions of the atmosphere to as much as 5% by volume in the hot humid air masses. The remaining gases are often referred to as trace gases, among which are the greenhouse gases such as $C0_2$ methane, nitrous oxide and ozone.

Many substances of natural origin may be present in locally and seasonally variable small amount as aerosols in an unfiltered air sample, including dust of minerals and microorganisms. There are lot of air borne particles that are major cause of respiratory ailments of humans, causing allergies, asthma and pathogenic infection of the respiratory tract. (Andrew et al., 2003).

The likes of *Mycobacterium tuberculosis, Mycoplasma pneumoniae, Streptococcus pneumoniae, Bordetella pertussis, Yersinia pestis* are responsible for different airborne diseases. Air can serve as a transport medium for different organisms that are capable of causing respiratory infections (Pepper et al., 2014).

Microorganisms in Air

In addition to gases dust particles and water vapour, air also contains microorganisms. Air can serve as a medium for transfer of microorganisms. These microorganisms present in the air can cause lot of diseases to human beings. If such air is inhaled by human beings, it can lead to some severe. Airborne microbes are biological airborne contaminants (also known as bioaerosols). They are bacteria, viruses, fungi as well as airborne toxins passed from one victim to another through air. Airborne microbes are the major cause of respiratory ailments such as allergies and pathogenic infections (Stetzenbach, 2009). Examples are as follows**:**

Viral diseases are as follows: Mumps, Influenza, Poliomyelitis, Common cold, Measles while the Causative agents are as follows respectively Mumps virus, *Mycovirus influence*, Poliovirus, Rhinovirus, Rubeola virus.

Bacterial diseases are as follows: Whooping cough, Diphtheria, Bronchitis, Atypical pneumonia, Pneumonic plague while the Causative agents are as follows respectively: *Bordetella Pertussis*, *Corynebacterium diphtheria, Hemophilus influenza, Mycoplasma pneumonia, Yersinia pestis.*

Fungal diseases are as follow Histoplasmosis, Cryptococcosis, Candidiasis, Blastomycosis while the Causative agents are as follows respectively *Histoplasma Capsulatum, Cryptococcus neoformans, Candida albicans, Blastomyces dermatitis*

The significance of air in microflora human health relies on the fact that air acts as a medium for the transmission of infectious agents. *Staphylococcus aureus* is the mostly commonly found in air since the carriers are commonly present (Madsen et al., 2018).

An abattoir also known as slaughter house is a place/facility where animals are killed for consumption as food products (Gram, 1996). Slaughtering animals on a large scale poses significant logistical problems and public health requirements. Public aversion to meat packaging in many cultures has certain conditions for the slaughtering of animals. There has been some criticism of the methods of transport, preparation, herding and killing in some slaughter houses.

In the 18^{th} and 19^{th} centuries in London, a combination of health and social concern led reformers to be concerned with the hygiene and disease as well as the effect of killing would have on the butchers, the observers and residents of areas they are situated. As a result of this tensions, meat markets within the city were also closed and also abattoirs built outside the city limits were closed (Tulchinsky,2014).

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Microorganism Present in Abattoir

Contamination of meat products by microorganism such as airborne bacteria and mold is a major economic problem in the meat industries. In the past it was thought that food products were contaminated surfaces, but now it is known that additional product occurs from contact with airborne bacteria and spoilage organisms can be introduced to meat products. It is known that contamination can occur at various points during the slaughtering process, cold storage and processing of meat animals. The airborne contaminants are also known as bioaerosols and include bacteria, fungi, and viruses. They may be present in air as solid and liquid (Jones et al., 2002). The majority of gram- negative airborne Caterair usually isolated during slaughtering are from the Enterobacteriaceae and Pseudomonadaceae families.

The gram-positive airborne bacteria that are usually isolated during slaughtering are staphylococcus, micro bacterium, bacillus and micrococcus species. The potential pathogenic micro-organisms usually found in the air sampling areas and on the carcasses are *Escherichia coli, Salmonella* spp, and *Bacillus* spp. The spoilage organisms are *Moraxella* spp, *Pseudomonas* spp, *Acinetobacter* s, *Micrococcus* spp (Sutton, 2014). There are some bacteria that are known for infecting meat while it is being processed, cut, packaged, transported, sold and handled. These are bacteria like *Escherichia coli, Bacillus proteus, Staphylococcus aureus, Bacillus cereus and Faucal streptococci.* Microbial population of the air can be done by passive monitoring using settle plates containing appropriate culture media that are open and expose for a given time and then incubated to allow visible colonies to develop and be counted. And active monitoring requires the use of a microbiological air sampler. It involves physically drawing a known amount of air over or through a particle collection device. The two main types are impactor and impingers.

Antibiotics

Antibiotics are a type of antimicrobial drugs used in treatment and prevention of bacterial infection. They may either kill or inhibit the growth of bacteria. Several antibiotics are also effective against fungi and protozoan and some are toxic to humans and animals, even when given in therapeutic dosage. Antibiotics are not effective against viruses such as the common cold or influenza and may be harmful when taken in-appropriately. Antibiotics revolutionized medicine in the 20^{th} century, and have together with vaccination led to the near eradication of disease such as tuberculosis in the developed world (Davies, 2010). Their effectiveness and easy access led to overuse, especially in livestock raising, prompting bacteria to develop resistance.

Classes of Antibiotics

Penicillin such as penicillin and amoxicillin

Cephalosporin such as cephalexin

Macrolides such as erythromycin, clarithromycin

Sulfonamides such as co-trimoxazole and trimethoprim

Tetracycline such as tetracycline and oxycline

Amino glycosides such as gentamycin and tobramycin

Studies have shown that there is growing concern of airborne affecting over 25% of population in urban civilized areas and with the estimate of about 50% of world population predicted to be affected by different disease as a result of human exposure to some microorganisms. With this development, there is therefore

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need to carry out assessment of microflora of some slaughter houses otherwise known as abattoirs in Akure metropolis of Ondo state, providing information about the occurrence, identity and reaction to antibiotics which justify the study. The major aim of this research is to determine the level of occurrence of isolate in a major abattoir (Onyaerugbulem) and a minor abattoir (FUTA), and also the antibiotic sensitivity patterns of airborne organisms present in both abattoirs

MATERIALS AND METHOD

Materials

Petri dishes, measuring cylinder, nutrient agar, MacConkey agar, distilled water, Bunsen burner, test tube rack, cotton wool, inoculating loop, slant bottles, triple sugar iron agar, cork borer, ethanol, retort stand, stirring rod, beakers, conical flask, citrate reagents, indole reagents.

Preparation and sterilization of culture media

5.5 grams of MacConkey agar, -6 grams of Eosin methylene blue agar, 2.8 grams of nutrient agar were weighed, dissolved with 100mls of water. Each of these media were sterilized in an autoclave at temperature of 121^{o}C for 15 minutes. Inoculation, pour plating and culturing were done near a naked flame from a bunsen burner to enhance septic condition (Fawole and Oso, 2007).

Isolation and Enumeration of Bacteria

Bacteria in the air were isolated using open plate method from two different abattoirs in Akure which are FUTA abattoir (minor) and Onyarugbule (major) abattoir. The Petri dishes containing sterilized prepared media were exposed for a period of 30minutes to different locations (on the roof of abattoir, slab floor, and on the table and benches). After exposure the plates, were brought back to the laboratory for incubation at a temperature of 37^{0}C for 24hours.

Sub culturing of bacteria

Colonies of different bacteria from agar media were streaked on freshly prepared nutrient agar. The plates were later incubated for 24hours and pure colonies of bacteria that were obtained from the sub cultured plates were later kept on agar slant.

Identification of bacteria

The appearance of colonies of the bacteria were studied and observed. Different characteristics were observed, which are colour, edge, shape, surface, elevation. After 24hours of incubation, the cultural characteristics, biochemical tests were obtained.

Biochemical tests

The following biochemical tests were carried out on the isolate: coagulase, citrate, indole, triple sugar iron, catalase and gram staining.

Gramstaining test

Gram staining also known as Gram's method of differentiating bacteria into two groups which are gram positive and gram negative bacteria was done. Gram positive bacteria appears purple because it contains the crystal violet dye while a counter stain added after crystal violet gives all gram negative red or pinkcoloring. Gram staining involves the preparation of smear from 24hours old culture. The smear on each of the slides

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was heated fixed by passing the backsides of the slides gently over the flame. The slides were stained with crystal violet and were left for 60 seconds, after which the slides were rinsed with water, and later stained with iodine and left 60 seconds, after that the slides were rinsed with water. After that ethanol was added onto each slide and were left for 30seconds before they were later rinsed with water. After that the slides wee later counter stained with safranin and were later left for 60seconds, after that the slides were rinsed with water and were allowed to dry. After drying a drop of immersion oil was added to each of the stained slides and were examined under oil immersion of the microscope as described by (Olutiola et al., 2000).

Catalase Test

Bacteria colonies were gotten from different subcultured plates and were placed on different cleanslides with the aid of sterile wooden sick. After that, several drops of hydrogen peroxide were placed onto the slides ad were mixed some slides gave positive result, this was known through rapid evolution of oxygen as evidenced by bubbling while others gave negative result since there was no bubble production.

Coagulase Test

A colony from the organism was emulsified in normal saline on grease free, clean slide and an equal volume of plasma were added and mixed together aseptically. Clumps or precipitate in the mixture indicates a positive coagulase test, this shows that the organism produces coagulase enzyme while the absence of clumps gives a negative result (Cheesbrough, 2006).

Indole Test

Indole test is a biochemical test performed on bacterial species to determine the ability of the organism to convert tryptophan into the indole. Peptone broth was prepared into different test tubes, and sterilized. After sterilization of the broth, the test tubes were inoculated with different organisms and later incubated for 24 hours at a temperature of 37^0C. After 4 hours, 2 drops of Kovac's reagent was put into each test tube containing peptone broth and organisms. The test tubes were made to stand for 20 minutes, after which different results were seen. Some gave red coloration, this as seen at the surface of broth, confirms that the result was positive while others gave yellow coloration, this confirms that the result was negative. Red coloration confirms positive results, while yellow coloration confirms negative result (Cheesbrough, 1998).

Citrate Test

The citrate test detects the ability of an organism to use citrate as the sole source of carbonand energy use of citrate involves the enzyme citrate, which break down citrate to oxaloacetate an acetate.

Simmon's citrate agar was prepared according to manufacture's instructions, and the agar was poured into each of the test tubes and allowed to gel. After that organisms were inoculated into the different labelled test tubes. All slants were incubated at 37^0C for 24 hours. After incubation some slants gave positive result, while others were negative, this is confirmed by the presence of growth on the surfaces of the slants which is accompanied by blue coloration. Citrate negative shows no growth and the medium remains green (Cappociano and Sherman, 1999).

Triple Sugar Iron Test

Triple sugar iron test is a microbiological test roughly named for its ability to ferment sugar and to produce hydrogen sulfide (Brown and Wiley, 2006). Triple sugar ironagar contains P^Hsensitive age, 1% lactose 1% sucrose 0.1% glucose as well as sodium thiosulfate and ferrous sulfate.

Ts1 slants were made, later the slants were inoculated with different organisms and incubated for 4hours at

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37^0C. Some changes were noticed, there was gas formation, acid production accompanied with H_2S in some of the slants, while some slants there was gas formation and acid production only, while in a particular one, there was no reaction. Acid production is noticeable through color change (Bayon and Thompson, 2001).

Antibiotic Sensitivity

Antibiotic sensitivity is the susceptibility of bacteria to antibiotics. Antibiotic susceptibility testing is usually carried out to determine which antibiotic will be successful in treating bacteria infection in vivo (Curtis, 2003). Testing for antibiotic sensitivity is often one by the Kirby-Bauer method. Peptone water was put into different test tubes, different organisms wee inoculated into the test tubes. The -peptone water containing different organisms was poured into different petri dishes containing freshly prepared nutrient agar. After some minutes the water was drained off, the plates were left to dry for some minutes, after that, antibiotic discs were placed on the plates with help of sterile forceps. The plates were later incubated at a temperature of 37^0C for 24hours. Organisms were susceptible to some antibiotics, that is zones of inhibition were seen and at the same time some organisms were resistant to some antibiotics.

RESULTS

The result from the various characterization, identification, and biochemical tests and antibiotic sensitivity carried out in the research are listed in the following tables.

Table 1.1: Morphological Characteristics of the Bacteria Isolated from the Sampled Abattoirs in Akure Metropolis.

The table below shows the morphological characteristics of the bacteria that were isolated from Onyaerugbulem and Futa abattoir. Shape, colour and elevation were observed.

SHAPES	COLOUR	ELEVATION	PROBABLE ORGANISMS
Cocci	Deep yellow	Raised	Staphylococcus aureus
Cocci	Light yellow	Raised	Micrococcus luteus
Rod	Cream	Raised	Bacillus spp
Rod	Yellow	Raised	Enterobacter aerogenes
Rod	Light yellow	Flat	Aeromonas spp
Rod	Cream	Flat	Proteus mirabilis
Rod	Cream	Raised	Escherichia coli
Rod	Cream	Flat	Proteus vulgaris

Table 1.2: Biochemical Tests of the Bacteria Isolated from the Sampled Abattoirs in Akure Metropolis.

The table below shows the biochemical tests that were carried out on the bacteria which were isolated. The tests are catalase, coagulase, citrate, indole, TSI, hydrogen sulphide formation and gram stain of these organisms gave positive and negative result.

NAMES OF ORGANISMS	CATALASE	COAGULASE	CITRATE	INDOLE	TSI	HYDROGEN SULPHIDE FORMATION	GRAM STAIN
Staphylococcus aureus *Micrococcus luteus*	+ve	+ve	+ve	-ve	*A&G*	-ve	+ve

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Bacillus spp *Enterobacteria aerogenes*	+ve	-ve	-ve	-ve	-ve	-ve	+ve
Aeromonas spp	+ve	-ve	+ve	+ve	*A&G*	-ve	+ve
Proteus mirabilis *Escherichia coli*	+ve	-ve	+ve	-ve	*A&G*	-ve	+ve
Proteus vulgaris	+ve	-ve	+ve	-ve	*A&G*	+ve	-ve
	+ve	-ve	-ve	-ve	*A&G*	+ve	+ve
	+ve	-ve	+ve	-ve	*A&G*	-ve	-ve
	+ve	-ve	-ve	-ve	*A&G*	+ve	-ve

KEY:

-ve = Negative reaction

+ve = Positive reaction

A&G = Acid & Gas Production

A = Acid Production

Table 1.3: Bacteria count Isolated from Onyaerugbulem Abattoir.

The table below shows the total count of bacteria that were isolated from different sample points of Onyaerugbulem abattoir. The sample points are gutter, sleeping bench, slaughter floor, slaughtering table and dump site.

Sample Point	**Total Bacteria count (CFU)**	**Total Coliform Count (CFU)**	**Total Escherichia coli Count (CFU)**
Gutter	255	35	36
Sleeping bench	156	41	43
Slaughter floor	142	28	65
Dump site	134	32	37
Slaughtering table	150	39	36

Table 1.4: Bacteria count Isolated from Futa Abattoir.

The table below shows the total count of bacteria that were isolated from different sample points of Futa abattoir. The sample points are roof, slaughter table and slaughter floor.

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Sample Point	Total Bacteria count (CFU)	Total Coliform Count (CFU)	Total Escherichia coli Count (CFU)
Roof	60	41	57
Slaughter table	52	34	58
Slaughter floor	156	43	72

Table 1.5: Bacteria Isolated from Onyaerugbulem Abattoir.

Area of the abattoir sampled	Types of organisms isolated
Slaughter slap	*Staphylococcus aureus, Proteus vulgaris, Bacillus spp, Escherichia coli, Aeromonas spp*
Sleeping bench	*Enterobacter aerogenes, proteus mirabilis*
Slaughtering table	*Staphylococcus aureus, Bacillus spp, Escherichia coli, Proteus mirabilis*
Dump site	*Escherichia coli, Aeromonas spp, Bacillus spp*
Gutter Edge	*Enterobacter aerogenes*

Table 1.6: Bacteria Isolated from Futa Abattoir.

Area of the abattoir sampled	Types of organisms isolated
Slaughtering slap	*Staphylococcus aureus, Aeromonas spp*
Roof top	*Escherichia coli,*
Slaughtering table	*Staphylococcus aureus, Proteus mirabilis*

Table 1.7: Antibiotic sensitivity pattern of gram Positive Bacteria from Onyaerugbulem and Futa Abattoir.

Organisms Antibiotic	Micrococcus luteus	Staphylococcus aureus	Bacillus spp
Pefloxacin	0.0	10mm	17mm
Gentamycin	12mm	22mm	20mm
Erythromycin	8mm	20mm	0.0
Ciprofloxacin	0.0	24mm	18mm
Streptomycin	0.0	0.0	0.0
Rocephin	14mm	12mm	14mm
Amoxicillin	0.0	0.0	0.0
Zinnacef	0.0	0.0	0.0
Ampiclox	15mm	0.0	0.0
Septrin	12mm	0.0	10mm

DISCUSSION

The result from this study shows the presence of different kinds of bacteria isolated from Onyaruegbulem and FUTA abattoir. A total of 8 bacteria were isolated comprising of both gram negative and gram-positive bacteria during the course of this research work. The bacteria isolated were *Escherichia coli*, *Bacillus* spp, *Proteus mirabilis Enterobacter aerogenes*, *Micrococcus* luteus, *Aeromonas* spp, *Staphylococcus aureus* and *Proteus vulgaris*. The presence of these organisms in the abattoir environment or their facilities that are

being used could be attributed to the fact that meat contains an abundance of nutrient required for the growth of microorganisms. *Escherichia coli* and *Bacillus* spp are the most prevalent organisms that were observed during the course of this research work. Microorganisms that were isolated in this study have been found earlier in foods, environment and other places as reported by Enubuele and Uraih, (2009). The presence of these organisms in the abattoir environment depicts the state of hygiene and sanitary practices employed during slaughtering process and packaging of meat. Most of the organisms found in this study are those commonly found in soil and water. The possible sources of contaminants could be due to the unhygienic manner of handling meat in abattoirs and environment where animals are slaughtered. This implies that such environment could be dangerous to human health if proper sanitization and hygiene is not maintained. The antibiotic sensitivity pattern was carried out. Ciprofloxacin antibiotic had the highest inhibitory effect on both gram positive and gram-negative bacteria (24 and 21 millimeters respectively), and thus could be recommended to workers that are exposed to these isolates.

CONCLUSION

In Conclusion, both major and minor abattoir considered in this study were heavily contaminated with bacteria. Thus, attention must be given to control those environmental factors which favor the growth and multiplication of microbes in the abattoir environment to safeguard the health of workers, observers and residents in those area. Also the management of the abattoirs needs to increase and properly carry out the sanitation of the abattoir so as to improve the quality of air being emitted from the area.

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