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Antimicrobial Susceptibility Pattern of Microorganisms Isolated from Abattoirs in Awka Metropolis, Anambra State, Nigeria

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ABSTRACT

The research was aimed at determining the sensitivity and/or resistance of isolated microbes from abattoirs to the various antimicrobial drugs used during food-borne illness caused by the consumption of abattoir-related products (such as meat) and the work-place related disease. The Amansea and Kwata abattoirs were used as study areas. Soil samples, wastewater effluents, swabs from cutting tables, equipments and slaughtered meat samples were aseptically collected from the abattoirs and the microbes were isolated using standard methods. The selected pure isolates were further identified as *Staphylococcus* species, *Streptococcus* species, *Candida albicans*, *Rhodotorula* species, *Rhizopus* species, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium notatum*. Using the standard disc diffusion method, the antimicrobial susceptibility pattern of isolates was determined against antibacterial agents (Rocephin (30µg), Pefloxacin (10µg), Gentamycin (10µg), Ciprofloxacin (10µg), Streptomycin (30µg), Ampiclox (30µg), Septrin (30µg), Zinnacef (20µg), Erythromycin (19µg), Amoxicillin (30µg)) and antifungal agents (Nystatin, Fluconazole, Ketoconazole). The effectiveness of the antimicrobials or the susceptibility of the isolates were defined in percentage as follows: Rocephin (7.7%), Pefloxacin (10.9%), Gentamycin (9.3%), Ciprofloxacin (8.8%), Streptomycin (10.4%), Ampiclox (9.5%), Septrin (11.0%), Zinnacef (10.7%), Erythromycin (11.8%), Amoxacillin (10.0%) (for antibacterial) and Nystatin (37.0%), Fluconazole (27.0%), Ketoconazole (36.0%) (for antifungal). Erythromycin and Nystatin can thus be recommended as the most effective antibacterial and antifungal agents respectively. Bacterial and fungal resistance was seen among drugs such as Rocephin and Fluconazole respectively.

Keywords

Abattoirs, Susceptibility
Pattern, Antimicrobial,
Microorganisms,
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Introduction

An antimicrobial agent is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoa (Michigan State University Board of Trustees, 2011). A successful antimicrobial agent must be able to

kill or inhibit the pathogen while causing little or no damage to the host. In other words, the microbial pathogen(s) must be susceptible to the drug. Susceptibility is the quality or state of being susceptible; which is lack of ability to resist some extraneous agent (as a pathogen or drug). Generally, the determination of

antimicrobial effectiveness against specific pathogens is essential to proper therapy. This is because sensitivity/susceptibility testing can show which antimicrobial drugs are most effective against a pathogen and also give an estimate of the proper therapeutic dosage (Michigan State University Board of Trustees, 2011).

An abattoir also known as a slaughterhouse is a building where animals are killed for their meat. It is an establishment where animals such as cattle, goats, sheep, pigs and others meat producing animals are butchered. Abattoir operation could be very beneficial to man; in that it provides meat for human consumption and other useful by-products, still it can be very hazardous to public health in respect to the waste it generates (Nandita *et al.*, 2015).

Diseases like ringworm, candidiasis, histoplasmosis, pneumonia, asthma, wool sorter diseases, respiratory and chest diseases were reported to be associated with abattoir activities (Nandita *et al.*, 2015).

Awka the capital of Anambra State of Nigeria and a pre-colonial city situates on latitude 6°02' 25" N and longitude 7°00'E.

It lies within the rainforest area but is now classified within the Guinea Savannah because of its derived vegetation, as the original vegetation has been removed by man. The mean annual rainfall is about 1524 mm with a relative humidity of 80% at dawn. Its location and its climatic characteristic high ambient temperature and rainfall add to the problem of waste management because of rapid rate of putrefaction with its attendant odor. Awka is about 40 km on the direct route from Onitsha with East–West extension of 8 km on Enugu-Onitsha express way corridor (Okonkwo, 2014). Amongst the various abattoirs in Awka metropolis, the two worked on with regards to

this research work are the Amansea abattoir and Kwata abattoir.

This study is aimed at determining the antimicrobial susceptibility pattern of microorganisms isolated from abattoirs in Awka metropolis, Anambra state. Thus, the significance of this research is to understand and figure out the sensitivity (effectiveness of an antimicrobial drug) and/or resistance of these isolated microbes to the various antimicrobial drugs used in times of the occurrence of foodborne illness caused by the consumption of abattoir-related products (such as meat) and the work-place related disease.

Materials and Methods

Sample collection

The observational and informative study was conducted between May and August 2017. Samples collected are soil samples, wastewater effluents, swabs from cutting tables, swabs from cutting equipments and swabs from slaughtered meat samples. Soil samples were collected in sterile containers using random sampling from four different locations in the different abattoirs. The sterile bottles were individually filled with wastewater effluents from the abattoirs, leaving a top space of about 2.5cm. A total of twelve (12) samples were collected individually from the two abattoirs from the cutting tables, cutting equipment and slaughtered meat using sterile swab sticks.

Samples were properly labeled by date of collection, name of the abattoir, and sample type. Containers with the soil and waste water samples and the swab sticks were properly sealed and transported to the laboratory of Applied Microbiology and Brewing Department, Nnamdi Azikiwe University, Awka for bacterial and fungal analyses immediately after collection.

Sterilization of materials

Proper sterilization of the sample containers, sample bottles and other materials were ensured before use.

Isolation of microorganisms

The soil samples collected each from Abattoir A (that is Amansea) and Abattoir B (that is Kwata) are properly mixed respectively and labeled soil sample A and B. Five (5) g of soil from each sample was transferred into 45 ml of sterile distilled water, and aseptically, serial dilution was performed to obtain soil suspension upto 10^{-6} . 0.1ml of each dilution (10^1 , 10^{-3} and 10^{-6}) was inoculated on Nutrient agar medium and Sabouraud dextrose agar using spread plate method. For the wastewater effluent samples, the same procedure was followed except 1.0 ml of the water was transferred (instead of 5 g of soil) into 9 ml of sterile distilled water for the 10^{-1} dilution. Note that plating out should be done in duplicate. The NA plates were incubated at 37°C for 18-24 hours, whereas the SDA plates were incubated at room temperature ($28\pm 1^\circ\text{C}$) 24-96 hours. Sub-culturing was done until distinct colonies (pure cultures) were obtained. Note in the isolation of these discrete colonies, streaking method was used for bacteria while stab inoculation was used for fungi. The discrete colonies were re-inoculated into appropriate media slants and were kept at 4°C for identification purpose (Nandita *et al.*, 2015).

The swab sticks containing the samples were streaked directly onto Nutrient agar and Sabouraud dextrose agar, and then incubated at 37°C and $28\pm 1^\circ\text{C}$ respectively for 24 hours for bacteria and 48 to 72 hours for fungi. Isolation of isolates to obtain discrete colonies was done. Note that, streaking method was used for bacteria while stab inoculation was used for fungi in the isolation of these discrete

colonies. The discrete colonies were re-inoculated into appropriate media slants and were kept at 4°C for identification purpose (Uzoma *et al.*, 2016).

Identification and confirmation of isolates

For bacteria, pure cultures were isolated. Their various cultural appearances were recorded, followed by microscopic and biochemical tests to identify the isolates. Microscopic and biochemical tests done using standard methods include; Gram staining, motility, catalase, coagulase, oxidase and indole. In identifying fungi, microscopic and macroscopic examinations including staining for morphological characteristics were carried out on fungal isolates. Identification was done based on the comparison of these characteristics using fungal atlas, so as to identify the fungi isolates to genus level.

The macroscopic colonial appearances of the pure fungal isolates are recorded. After which, wet mount- lactophenol staining of the isolates are done on the slides; the characteristic features viewed are recorded also. These results are compared with the colonial morphologies of identified fungi in the fungal atlas in order to identify the pure fungal isolates. Note, various pictorial images of identified fungi colonies on Google also proved helpful in the identification.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing on all the isolates was done using disc diffusion method. For bacterial isolates, sensitivity disks containing conventional antimicrobials which are Pefloxacin ($10\mu\text{g}$), Gentamycin ($10\mu\text{g}$), Ampiclox ($30\mu\text{g}$), Zinnacef ($20\mu\text{g}$), Amoxicillin ($30\mu\text{g}$), Rocephin ($30\mu\text{g}$), Ciprofloxacin ($10\mu\text{g}$), Streptomycin ($30\mu\text{g}$), Septrin ($30\mu\text{g}$) and Erythromycin ($19\mu\text{g}$). Nystatin, Fluconazole and Ketoconazole were

used for the sensitivity test against the fungal isolates. The pure bacterial and fungal isolates are evenly streaked individually on the appropriate agar plates that are properly labeled. After which, the inoculums were dried at room temperature for 30 min, then antibiotic impregnated disks were applied to the surface of the inoculated plates using sterile forceps. Note; the sensitivity discs containing antibacterial drugs were placed on the surface of each Nutrient agar plate evenly seeded with test bacterial organisms and was incubated for 24 h at 37°C while the sensitivity discs containing antifungal drugs were placed on the surface of each Sabouraud dextrose agar plate evenly seeded with test fungal organisms and was incubated for 24-72 h at 25°C -27°C (Bauer *et al.*, 1996).

Measurement of zone of inhibition

After 24 hours of incubation in the case of bacteria and 48-72 hours incubation in the case of fungi, the antimicrobial sensitivity results for each isolates against each antimicrobial drug was read by measuring the zones of inhibition using ruler. The diameter of the zone were measured and recorded; that is for organisms susceptible to the antimicrobials.

Isolates that was not susceptible/ sensitive to the drugs showed no zone of inhibition and are thus termed resistant to the antimicrobial agents.

Results and Discussion

Mycological count of the fungal isolates

Mycological count of the fungal colonies isolated from samples collected from Amansea and Kwata abattoirs were recorded respectively on the 25th of May (2 days after inoculation onto the agar plate). For Amansea samples at this time, the soil sample of 10⁻¹

dilution had the highest number of colonies (40 colonies) while the meat swabs had no growth yet. For Kwata samples at this time, the meat swabs had the highest number of colonies (298 colonies) while soil sample of 10⁻¹ dilution had no growth yet. The results are represented below using bar charts in figures 1 and 2.

Morphological characteristics of the selected isolates from the Abattoir samples

From the various bacterial and fungal colonies isolated from the studied samples, six (6) bacteria and six (6) fungal isolates were selected for further identification and antimicrobial sensitivity testing.

The morphological characteristics of bacteria considered are size, shape, color, elevation, margin, surface and transparency while those considered for the fungi are the fungal type, color, underside color, colony age, growth rate and colony texture. The results are shown in the tables 1 and 2.

Microscopic and biochemical tests for microbial identification

The microscopic analyses showed that all the bacterial and yeast isolates were Gram positive. Most bacterial isolates were cocci except isolate 6 which was diplococci.

The results of the various microscopic and biochemical tests done on the bacterial and fungal isolates are shown in tables 3 and 4.

Probable organisms suspected

The results of the colonial observations, microscopic and biochemical tests carried out enabled the probable identification of the bacterial and fungal isolates, which were represented in tables 5 and 6.

Table.1 Morphological characteristics of the selected bacterial isolates

Isolate	Size (mm)	Shape	Color	Elevation	Margin	Surface	Transparency
1	8	Circular	Creamy	Raised	Entire	Concentric	Opaque
2	3	Irregular	Creamy	Flat	Undulated	Smooth	Opaque
3	1.5	Circular	Brown	Flat	Entire	Smooth	Opaque
4	Puntiform	Circular	Yellow	Raised	Entire	Glistening	Opaque
5	1	Circular	Orange	Flat	Entire	Smooth	Opaque
6	2.5	Circular	Yellow	Raised	Entire	Dull	Opaque

Table.2 Morphological characteristics of the selected fungal isolates

Isolate	Fungal type	Color	Underside color	Colony age (days)	Growth rate	Colony texture
1	Yeast	White	Creamy	3	Moderate	Contoured
2	Yeast	Pink	Pink	2	Rapid	Smooth
3	Mold	White	Orange	2	Slow	Cottony
4	Mold	Black(center) and White(side)	Brown(center) and Yellow(side)	2	Rapid	Cottony
5	Mold	Green(center) and White(side)	Yellowish-green	2	Rapid	Cottony
6	Mold	Green(center) and White(side)	Yellow	2	Slow	Wrinkled

Media used: Sabouraud dextrose agar (SDA)

Table.3 Microscopic and Biochemical tests for the identification of the bacterial isolates

Isolate	Gram staining	Rod / Cocci	Motility test	Catalase Test	Coagulase test	Oxidase test	Indole test
1	+	Cocci	-	-	-	-	-
2	+	Cocci	+	+	+	-	-
3	+	Cocci	-	+	-	-	-
4	+	Cocci	-	+	-	-	-
5	+	Cocci	-	+	-	-	-
6	+	Diplococci	-	+	-	-	-

Table.4 Microscopic and biochemical tests for the identification of the fungal isolates

Isolate	Gram staining	Wet mount-Lactophenol observations
1	+	Circular buds, pseudohyphae
2	+	Oval buds
3		Aseptate hyphae, blastospores
4		Aseptate hyphae, sporangium, sporangiospores, sporangiophores
5		Aseptate hyphae, clustered conidiospores, conidiospores
6		Aseptate hyphae, sporangiospores, sporangium, sporangiophores

Table.5 The bacterial isolates

Isolate	Probable bacterial organism
1	<i>Streptococcus</i> species 1
2	<i>Staphylococcus aureus</i>
3	<i>Staphylococcus</i> species 1
4	<i>Staphylococcus</i> species 2
5	<i>Staphylococcus</i> species 3
6	<i>Streptococcus pneumonia</i>

Table.6 The fungal isolates

Isolate	Probable fungal organism
1	<i>Candida albicans</i>
2	<i>Rhodotorula</i> species
3	<i>Rhizopus</i> species
4	<i>Aspergillus niger</i>
5	<i>Aspergillus flavus</i>
6	<i>Penicillium notatum</i>

Table.7 The zone of inhibitions (cm) shown by the bacterial isolates

Isolate	R	CPX	S	SXT	E	PET	CN	APX	Z	AM
1	1.35	1.5	1.4	1.5	1.7	1.5	1.3	1.25	1.5	1.2
2	1.45	1.5	1.6	1.6	1.8	1.5	1.6	1.8	1.8	1.45
3	1.4	1.0	1.5	1.8	1.8	1.75	0.8	1.25	1.6	1.4
4	R	0.9	1.6	1.5	1.5	1.6	1.5	1.65	2.0	2.0
5	1.3	1.4	1.2	1.6	1.9	1.7	1.5	1.4	1.5	1.3
6	1.5	1.65	2.1	2.0	2.0	1.8	1.75	1.25	1.3	1.75

Key- R: Rocephin (30µg) PEF: Pefloxacin (10µg)
 CN: Gentamycin (10µg) CPX: Ciprofloxacin (10µg)
 S: Streptomycin (30µg) APX: Ampiclox (30µg)
 SXT: Septrin (30µg) Z: Zinnacef (20µg)
 E: Erythromycin (19µg) AM: Amoxicillin (30µg)
 (R) in the results: Resistant

Table.8 The zone of inhibitions (cm) shown by the fungal isolates

Isolate	NYS	FLU	KET
1	1.6	3.4	1.8
2	2.2	1.4	2.6
3	2.4	3.0	2.2
4	3.0	R	2.2
5	2.1	1.1	0.8
6	1.0	R	2.6

Key- NYS: Nystatin KET: Ketoconazole
 FLU: Fluconazole (R) in the results: Resistant

Figure 1: Mycological count of the fungal isolates from Amansea abattoir

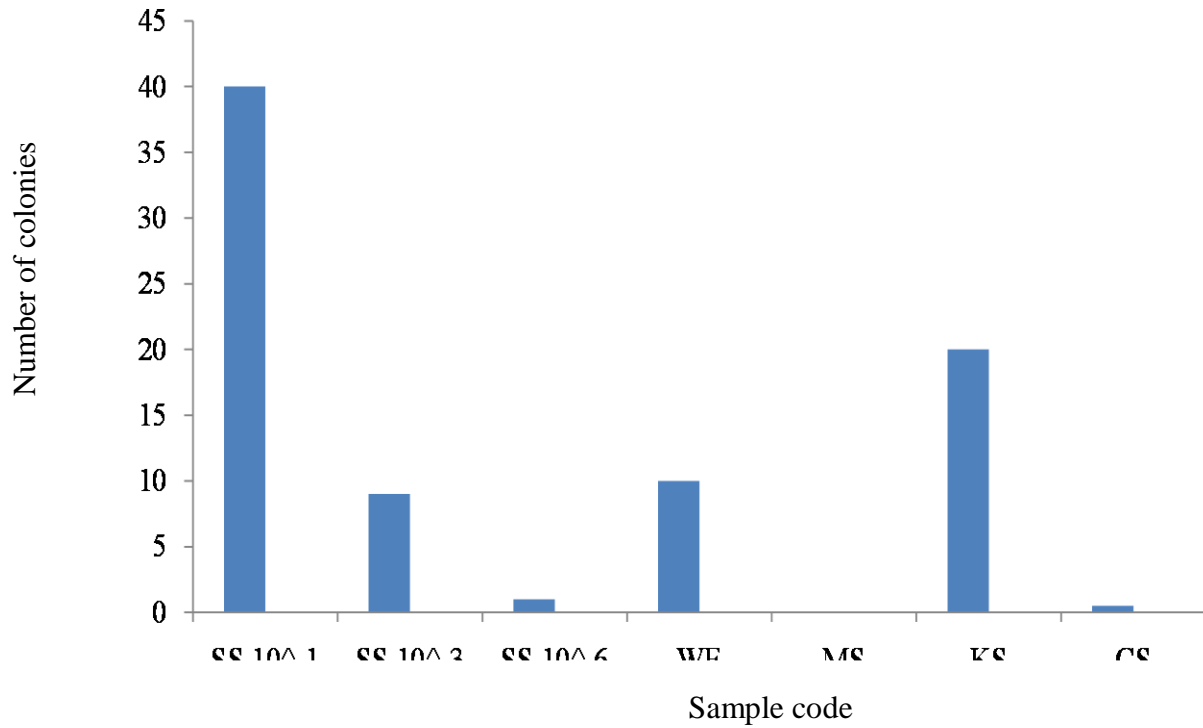
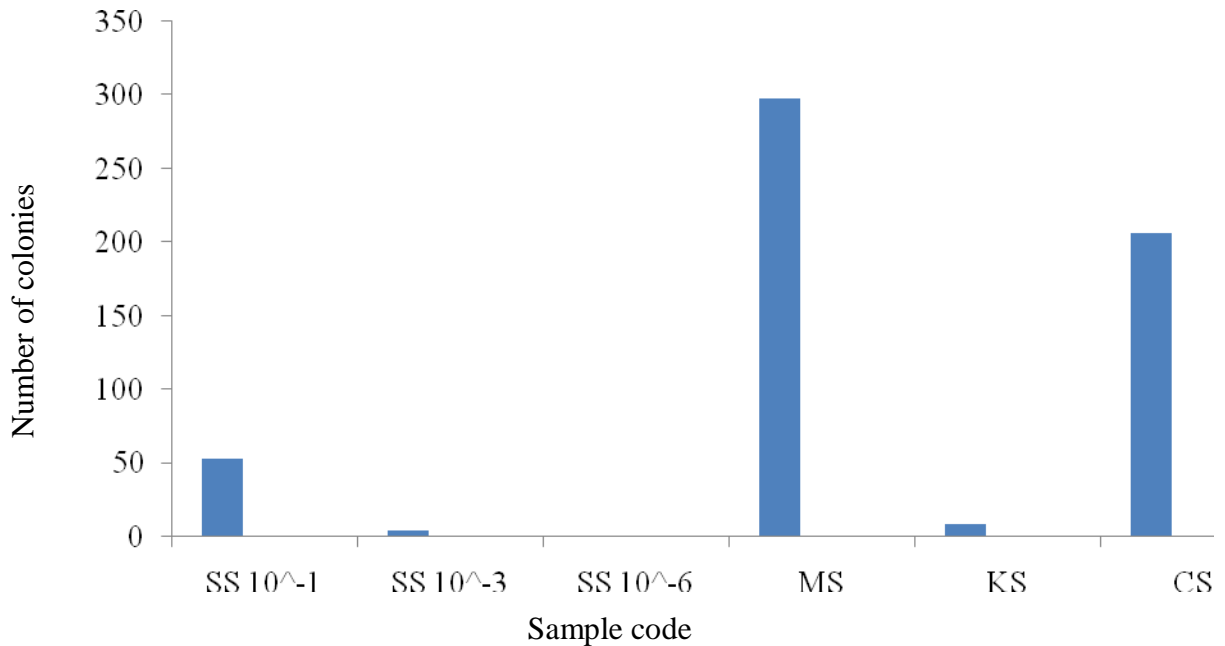
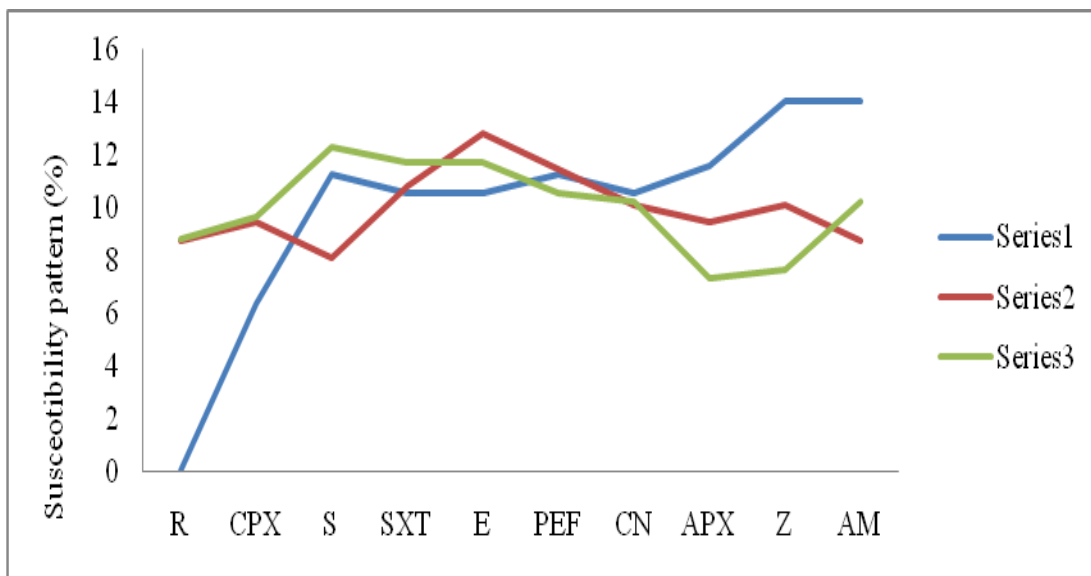


Figure 2: Mycological count of the fungal isolates from Kwata abattoir



Key – SS: Soil sample, WE: Wastewater effluent, MS: Swabs from slaughtered meat, KS: Swabs from cutting knife, CS: Swabs from cutting table

(a)

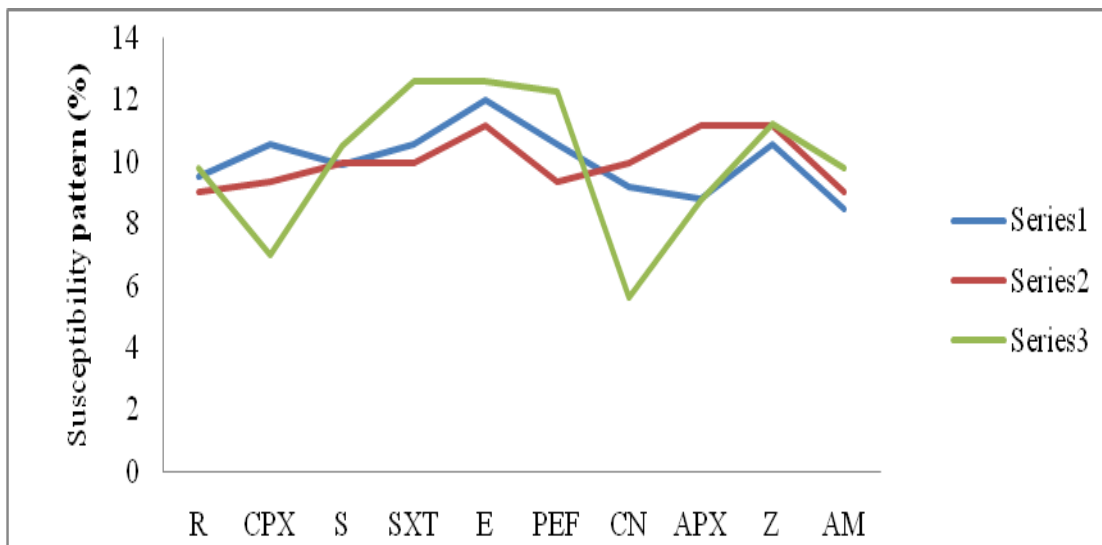


Key: Series 1= Isolate 1

Series 2= Isolate 2

Series 3= Isolate 3

(b)



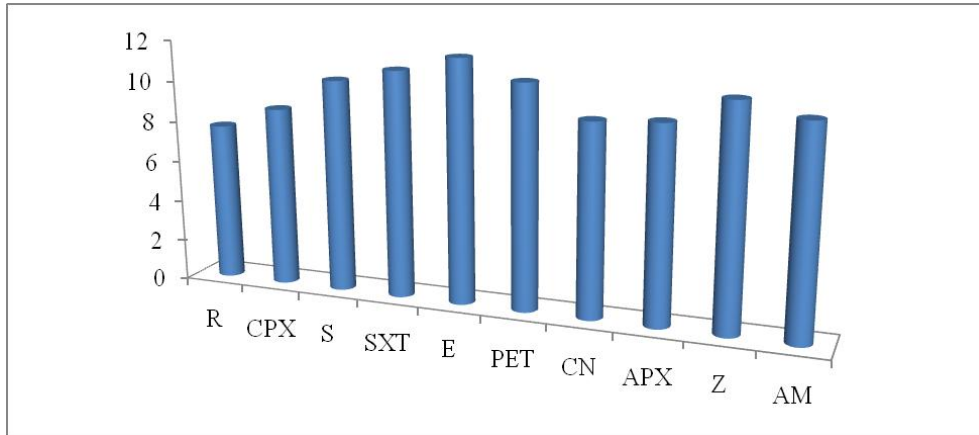
Key: Series 1= Isolate 4

Series 2= Isolate 5

Series 3= Isolate 6

Fig.3 (a) & (b) Antimicrobial susceptibility pattern (in %) of each bacterial isolate against the test antibiotics

Fig.4 Comparison (in %) of each of the test antibiotics/ antibacterial



Key: Series1= Antibiotic strength (%)

Fig.5 Antimicrobial susceptibility pattern (in %) of each fungal isolate against the test (antifungal)

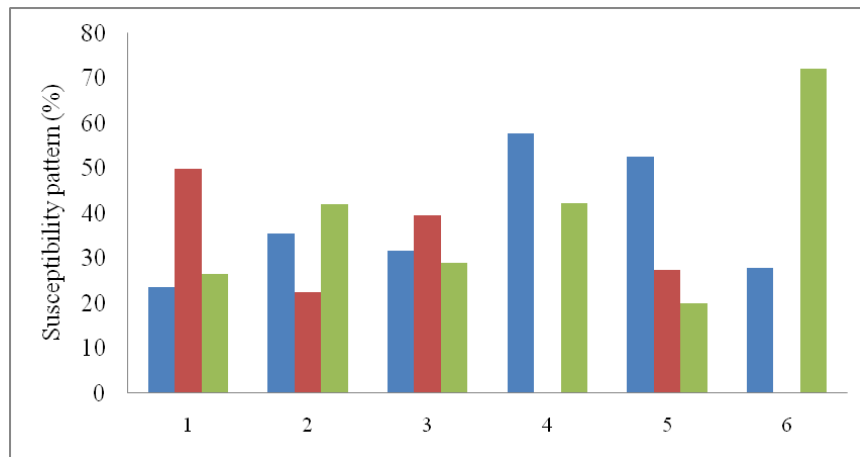
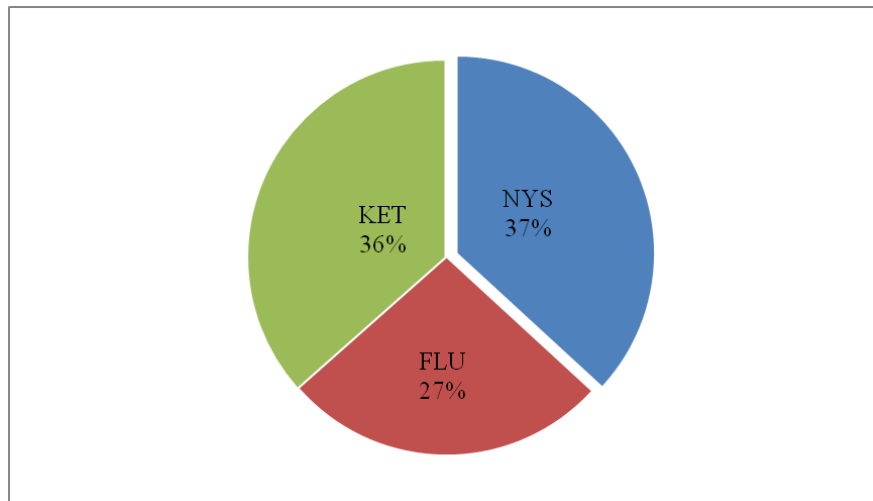


Fig.6 Comparison (in %) of each of the test (antifungal)



Antimicrobial sensitivity results for the identified isolates from the abattoir samples

The antimicrobial sensitivity results showed that the antibacterial: Erythromycin gave the highest collective zones of inhibition, followed by Septrin while Rocephin gave the least collective zones of inhibition.

In the case of antifungals, Nystatin gave the highest collective zones of inhibition while Fluconazole gave the least (Fig. 3 and 5). Antimicrobial sensitivity results are explicitly shown in tables 7 and 8.

Analysis of the antimicrobial sensitivity results

The antibacterial susceptibility patterns (%) of the bacterial isolates are as follows:

Isolate 1= R (9.5%), CPX(10.6%), S(9.9%), SXT(10.6%), E (11.9%), PEF(10.6%), CN(9.2%), APX(8.8%), Z(10.6%), AM(8.5%)

Isolate 2= R (9.0%), CPX(9.3%), S(9.9%), SXT(9.9%), E (11.2%), PEF (9.3%), CN(9.9%), APX(11.2%), Z(11.2%), AM(9.0%)

Isolate 3= R (9.8%), CPX(6.9%), S(10.5%), SXT(12.6%), E (12.6%), PEF(12.2%), CN(5.6%), APX (8.7%), Z(11.2%), AM(9.8%)

Isolate 4= R (0%), CPX(6.3%), S(11.2%), SXT(10.5%), E (10.5%), PEF(11.2%), CN(10.5%), APX(11.6%), Z(14.0%), AM(14.0%)

Isolate 5= R (8.8%), CPX(9.5%), S(8.1%), SXT(10.8%), E(12.8%), PEF(11.5%), CN(10.1%), APX(9.5%), Z(10.1%), AM(8.8%)

Isolate 6= R (8.8%), CPX (9.6%), S (12.3%), SXT (11.7%), E (11.7%), PEF (10.5%), CN (10.2%), APX (7.3%), Z (7.6%), AM (10.2%)

These antimicrobial susceptibility patterns are represented and compared using the line graph below:

The antifungal susceptibility patterns (%) of the fungal isolates are as follows:

Isolate 1= NYS (23.5%), FLU (50.0%), KET (26.5%)

Isolate 2= NYS (35.5%), FLU (22.6%), KET (41.9%)

Isolate 3= NYS (31.6%), FLU (39.5%), KET (28.9%)

Isolate 4= NYS (57.7%), FLU (0.0%), KET (42.3%)

Isolate 5= NYS (52.5%), FLU (27.5%), KET (20.0%)

Isolate 6= NYS (27.8%), FLU (0.0%), KET (72.2%)

These patterns are represented and compared using the clustered column graph below:

The morphological characteristics and the results of the microscopic and biochemical tests of the selected bacterial isolates suggested the probable bacterial organisms as *Staphylococcus aureus*, other *Staphylococcus species*, *Streptococcus pneumonia* and other *Streptococcus species*. On the other hand, the morphological characteristics and the results of the microscopic and biochemical tests of the selected fungal isolates suggested the probable fungal organisms as *Candida albicans*, *Rhodotorula species*, *Rhizopus species*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium notatum*. The microbes

isolated in this work were in agreement with the research of Nandita *et al.*, (2015).

Antimicrobial susceptibility/sensitivity patterns of these organisms were determined using specific antibiotics (Rocephin, Pefloxacin, Gentamycin, Ciprofloxacin, Streptomycin, Ampiclox, Septrin, Zinnacef, Erythromycin, Amoxicillin) and antifungal (Nystatin, Fluconazole, Ketoconazole), some of which were used by Anyim *et al.*, (2014), Nandita *et al.*, (2015), Nwiyi *et al.*, (2012), Rabirab *et al.*, (2014) and Tijani *et al.*, (2017).

For the antibiotics: Isolate 1 was more susceptible to Erythromycin but least susceptible to Amoxicillin. Isolate 2 was more susceptible to Erythromycin, Ampiclox and Zinnacef but least susceptible to Rocephin and Amoxicillin. Isolate 3 was more susceptible to Septrin and Erythromycin but least susceptible to Gentamycin. Isolate 4 was more susceptible to Zinnacef and Amoxicillin, least susceptible to Ciprofloxacin and resistant to Rocephin. Isolate 5 was more susceptible to Erythromycin but least susceptible to Streptomycin. Finally, Isolate 6 was more susceptible to Septrin and Erythromycin but least susceptible to Zinnacef. These susceptibility patterns are in agreement with Nandita *et al.*, (2015). Furthermore, considering the comparison (in %) of each of the test antibiotics as seen in figure 4, Erythromycin is the most effective antibiotic drug to be used in the case of abattoir related infection/ disease cases caused by these aforementioned bacterial isolates.

For the antifungal: Isolate 1 was more susceptible to Fluconazole but least susceptible to Nystatin. Isolate 2 was more susceptible to Ketoconazole but least susceptible to Fluconazole. Isolate 3 was more susceptible to Fluconazole but least susceptible to Ketoconazole. Isolate 4 was

more susceptible to Nystatin, least susceptible to Ketoconazole but resistant to Fluconazole. Isolate 5 was more susceptible to Nystatin but least susceptible to Ketoconazole. Finally, Isolate 6 was more susceptible to Ketoconazole, least susceptible to Nystatin but resistant to Fluconazole. Furthermore, considering the comparison (in %) of each of the test antifungal as seen in figure 6, Nystatin is the most effective antifungal drug to be used in the case of abattoir related infection/ disease cases caused by these aforementioned fungal isolates.

Despite its importance, abattoirs as seen in this work and many other related works, are niche for countless microorganisms of medical importance. As earlier stated, these pathogenic microbes could be the animals' normal flora or could be present as a result of poor sanitary, health and hygiene practices during meat handling, slaughtering, processing, distribution and even consumption. Thus, these is a wake-up call for these abattoirs to strategically implement strict sanitary operating procedures so as to reduce the microbial load of their end products and also reduce the risk of infection of its personnel. Furthermore, meat and other abattoir related products consumers should ensure that these products are properly cooked and processed before consumption. With respect to the antimicrobial susceptibility patterns of the isolates, results showed that most of the test antimicrobials were effective against the microbes, with Erythromycin and Nystatin being the most effective for the bacteria and fungi isolates respectively. Thus, Erythromycin and Nystatin should be the first line of prescription in cases of abattoir related infection and disease. It should be noted that bacterial and fungal resistance was seen among drugs such as Rocephin and Fluconazole respectively.

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