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Bacteriological Identification between Human and Sheep Living in the Same Shelter: Cyuve Sector, Musanze, Rwanda

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Abstract:

Background: *Domestic animals can spread infection to human and these infections known as zoonotic infections.*

Objectives: *The aim of this study was to identify common bacteria shared between human and sheep and their antibiogram. The following objectives were formulated; to determine the incidences common shared bacteria between sheep and human and to determine antibiotic sensitivity testing among bacterial isolates from humans and sheep.*

Methods: *Urine specimens were collected from both human and sheep and transported following three package system. Culture, gram stain, biochemical test and antibiogram test were performed. The obtained data were analyzed by using Statistical Package for Social Science (SPSS).*

Results: *The results from this study confirm that gram negative bacteria were highly frequent for sheep more than human with 70 % and 36.6 % respectively. Gram negative bacteria were more isolated in sheep where, Escherichia coli and Pseudomonas ssp were isolated with 43.3 and 26.6 % respectively. Among human, Escherichia coli and Pseudomonas ssp were isolated with 26.6 and 10 % respectively. Staphylococus aureus was the only gram positive bacteria isolated for both human and sheep with 23.3 and 13.3% respectively. Vancomycin and Ciproflaxacin were the antibiotics have high sensitivity to E. coli, Pseudomonas ssp and S. aureus. Pseudomonas ssp was also sensitive to Erythromycin. According to the results of this study, objectives were achieved and hypotheses conformed and verified with statistical significance of Pearson Chi-Square = 9.000, Df =1 P=0.003.*

Conclusion: *In fact, bacteria isolated from both sheep and human. Thus, animals could live in farming away from human settlements.*

Keywords: *antibiotics, Bacteria, resistance, sensitivity*

1. INTRODUCTION

Mankind lives in complex environment, and cooperates with other livings to survive in its environment. Domestic animals significantly play an immense role for human lives, and can serves as source of food, Money income and others (1). Despite positive impact of animals on human life, the interdependence between animals, human and environment can be an exposure to serious diseases disturbing public health as well as social economic and wellbeing of human population (2). The diseases transmitted from animals to humans are known as zoonoses and are transmitted through direct contacts or through water, food, and environmental conditions (3).

the bacteria Ana-plasma phagocytophilum is an etiologic agent of Tick-borne fever which is an endemic disease of sheep and cattle in tick (Ixodesricinus) infested areas of Norway. This infection has been reported in humans and number of domestic animals as well as wild animals in the region (4). TBF diseases is fatal and it can be aggravated and complicated by second infections on the patient. TBF is a leading cause of suppressed immunity (5). Sheep living with this infection are vulnerable to secondary infections like tick pyaemia caused by Staphylococcus spp. And Pasteurella (Mannheimia) septicemia. Complications also include abortion in pregnant ewes, reduction of milk

yield production in cattle, impaired spermatogenesis in rams, and reduction of weight gain in lambs (6).

Inframammary infections (IMI) is found mostly in dairy small ruminants, and are due to bacteria like Staphylococcus aureus (S. Aureus) and Streptococcus agalactiae (S. Agalactiae) or environmental pathogens (Escherichia coli), Pseudomonas aeruginosa, Streptococcus uberis and coagulase-negative staphylococci (7). Mastitis is an inflammation of breast or udder due to bacterial infections and can be treated using antibiotics after identifying etiologic agent of this disease (8). Antibiotic susceptibility test should be done to ensure adequate and correct treatment of the infection. The treatment of inframammary by means of antibiotic therapy using a combination of penicillin, nafcillin, and dihydrostreptomycin has been found to be more effective in reducing the load of mastitis pathogens after lambing in sheep (9). It is significantly important to owe the huge role of antimicrobials to strengthen mastitis control program, but also the determination of antimicrobial susceptibility of mastitis pathogens is necessarily needed to reinforce therapy and monitor the spreading of resistance (10).

Zoonotic diseases are known to be serious contagious diseases spreading from animals to humans. The estimation shows that approximately 75% of recent infectious diseases facing humans were from animal sources (11). Human can get infected via direct contact with animal which is infected, but also through consuming infected foods and water, inhalation, arthropod vectors and pests (12). Human also can get these infections through raw milk and cheese consumption but also products made from them can spread infections. Remember that most of fresh cheese are from sheep and goats (13). Zoonotic diseases are also known to be occupational diseases specifically for those whose daily work is in the livestock sectors (14).

Today, modern transportation has increased where both humans and animals are transported easily. This rises a public health burden because pathogens can be transported from one country to another in unnoticed ways, the pathogen can be with human, animal, plant and food transport and may reach one country to another within less than a day. This highest level of human and animal mobility makes difficulties in zoonotic pathogen control, it can require to close international collaboration as first interventions, however, ecological understanding is necessary to fight against international spreading of infections (15).

Zoonotic diseases or Zoonoses are numbers of communicable diseases that are transmitted from animals to humans and list the etiologic agents that cause Zoonotic diseases (16). These diseases are transmitted either by the consumption of contaminated food and water, exposure to the pathogen during preparation, processing or by direct contact with infected animals or humans (17). Statistically, it is evident that the prevalence and incidence of zoonotic diseases is a major public health problem worldwide. 58% to 61% of all communicable diseases causing illness are zoonoses constituted in human's worldwide (18).

With today's rapid transport systems, modern public health problems are growing increasingly complex. A pathogen that emerges today in one country can easily be transported unnoticed in people, animals, plants, or food products to distant parts of the world in less than 24 hours. This high level of mobility makes tracking and designing interventions against emerging pathogens exceedingly difficult, requiring close international and interdisciplinary collaborations. Fundamental to these efforts is an understanding of the ecology of emerging diseases (15-18).

2. METHODS AND MATERIALS

2.1. Study Design

This is being a cross sectional study. The population of interest were both humans and shelter living in the same shelter. Urine samples were collected on both humans and sheep and microbiological analysis was done to identify common shared bacteria between human and sheep. On both sides, 30 samples were taken and each one analysed independently. Purposive sampling was used because criteria of taking sample was addressed to human living with sheep and who accepted to participate in the study.

2.2. Specimens Collection

Urine specimens were collected from both humans and sheep. Appropriate sterile containers were used to collect specimens. The collected specimens were urine form both human and sheep and each

container used one time on both sides. The collected specimens were triple packaged and transported to INES-Ruhengeri microbiology laboratory for further analysis.

2.3. Sample Processing and Analysis

2.3.1. Preparation of Culture Media and Inoculation of Specimen

Cled Agar, MSA and BA were important media used for urine specimens culture, and SOP protocol was followed for media preparation. the use of streak plate technique, facilitated the inoculation of specimens in petri dish containing culture medium. All inoculated specimens were incubated for 24 hours, and after 24 hours, the growth of colonies were visible and changes in color was taken into consideration.

2.3.2. Gram Staining of Isolated Bacteria

Gram staining was used to differentiate gram positive and gram negative bacteria, this was done by using different dyes which are crystal violet, iodine, alcohol, fuchsine or Safranin. Gram positive retain the primary dye which is a crystal violet and Gram negative retain the last color which is fuchsine. Microscopically Gram positive appear in blue or purple color while Gram negative stained in pink color.

2.3.3. Biochemical Test

After incubation at 37oC, bacterial growth on culture media was presented and visible differently through colonial morphology characteristics. Based on the consistence, color, shape and size, the type of bacteria on culture media was suspected. In this study catalase and coagulase tests were used for differentiating Staphylococcus species and Streptococcus species gram positive bacteria.

2.4. In Vitro Antimicrobial Susceptibility Tests

The identified bacteria were Escherichia coli, Psedomonas ssp and Staphylococcus aureus and were all taken for antibiotic susceptibility test. Each antibiotic test considered the origin of each bacterium, whether from humans or animals. Antibiotics used were Vancomycin (VA), Ciproflaxacin (CIP), Cefuroxime (CXM), Nitrofuranton (F), Clindamycin (DA) and Erythromycin (E). Resistant and sensitivity were measured by referring to the standard of each antibiotic.

The suspension was well spread aseptically on Miller Hinton culture media used for antimicrobial susceptibility test by using sterile swab. Different antibiotic discs were then put on the surface of inoculated plate and incubated between 18-24 hours at 37oC. The inhibition zones were then measured and interpreted disc diffusion method of bacteria where it was possible to determine if bacteria were resistant or sensitive on different antibiotic disc used.

2.5. Statistical Analysis

The statistical package for social sciences (SPSS) version 22 was used for data analysis. The data was presented in form of tables and figures.

2.6. Ethical Consideration

The specimens used to reach the results of this study were collected after getting written permission of data collection signed by Mayor of Musanze district. No records of like names, sex and personal deification were recorded for this study.

3. RESULTS INTERPRETATION

3.1. Identification of Bacteria by Gram Staining

Bacteria could be identified by gram stain after culture. The following table presents identification of bacteria from both human and sheep.

Table1. *Bacteria identification from both human and sheep*

	Total	Gram + bacteria	Percentage (%)	Gram - bacteria	Percentage (%)
Human	30	7	23.3	11	36.6
Sheep	30	4	13.3	21	70
Sheep	30	4	13.3	21	70

Pearson Chi-Square = 9.000, Df =1 P=0.003

According to the table above, gram negative bacteria were highly frequent for sheep more than human with 70 % and 36.6 % respectively. There is statistical significance showing that there is a share of bacteria between human and sheep, and this justify that they can also share infection in case of diseases among them (P>0.5, p=0.003). This can be an indicator of the effect of human bacterial infection resulting from living with sheep.

3.2. Types of Isolated Bacteria

The table below present's bacteria isolated among human and sheep living in the same shelter in Cyuve sector.

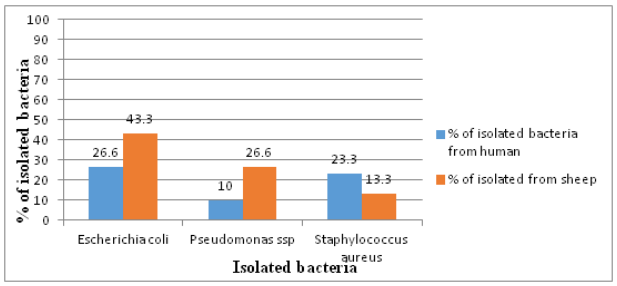

Figure1. *Types of isolated bacteria and their percentages*

Common bacteria shared between human and sheep are shown on the figure above. According to the results from figure above, bacteria were more isolated in sheep than in human. Gram negative bacteria were more isolated in sheep where, Escherichia coli and Pseudomonas ssp were isolated with 43.3% and 26.6 % respectively. Among human, Escherichia coli and Pseudomonas ssp were isolated with 26.6% and 10 % respectively. Staphylococcus aureus was the only gram positive bacteria isolated for both human and sheep with 23.3 and 13.3% respectively. The isolation of bacteria at high level in sheep can be a key factor justifying the capacity of sheep to transmit bacteria to human.

3.3. Susceptibility of Isolated Bacteria to Antibiotics

Bacteria have different capacity of resistance to antibiotics. The following figure presents sensitivity and resistance of isolated bacteria from human and sheep to antibiotics. Antibiotics used were Vancomycin (VA), Ciproflaxacin (CIP), Cefuroxime (CXM), Nitrofuranton (F), Clindamycin (DA) and Erythromycin (E).

R stands for Resistant and S is sensitivity.

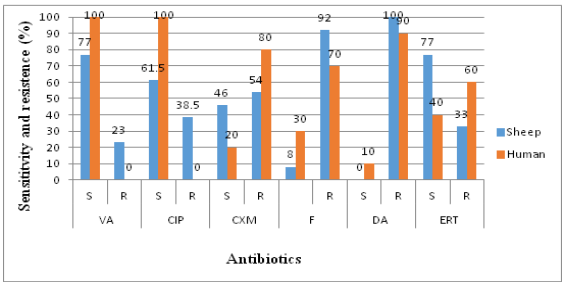

Figure2. *Resistance of human and sheep E. coli to antibiotics*

The figure 2 shows that for human; vancomycin and ciproflaxacin were sensitive to E. coli 100%. Cefuroxime was sensitive to E. coli 20% and E. coli was resistant to Cefuroxime 80%. Nitrofuranton was sensitive to E. coli by 30% while E. coli was resistant to Nitrofuranton on 70%. Clindomycin was sensitive to E. coli 10% while E. coli was resistant to clindomycin 90%. Erythromycin was sensitive to E. coli 40% and E. coli was resistant to Erythromycin 60%.

figure above also shows that for sheep; vancomycin was sensitive to E. coli 77% and E. coli was resistant to vancomycin 23%. Ceproflaxacin was sensitive to E. coli 61.5% while E. coli was resistant to Ceproflaxacin38.5%. Cefuroxime was sensitive to E. coli 46% and E. coli was resistant to Cefuroxime 54%. Nitrofuranton was sensitive to E. coli by 8% while E. coli was resistant to Nitrofuranton on 92%. E. coli was resistant to clindomycin 100%. Erythromycin was sensitive to E. coli 77% and E. coli was resistant to Erythromycin 33%.

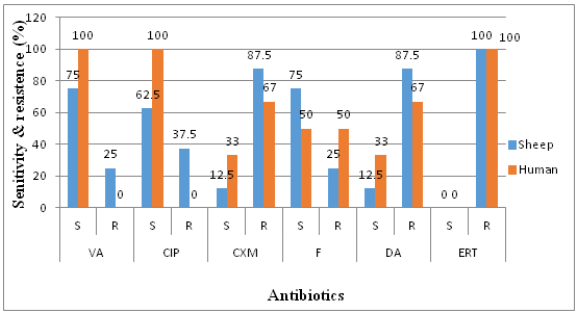

Figure3. *Resistance of human and sheep Pseudomonas ssp to antibiotics*

The figure 3 shows that for human; vancomycin and ciproflaxacin were sensitive to *pseudomonas ssp* 100%. Cefuroxime was sensitive to *pseudomonas ssp* 33% and *pseudomonas ssp* was resistant to Cefuroxime 67%. *Pseudomonas ssp* was resistant to Nitrofuranton 50% and Nitrofuranton was sensitive to *pseudomonas ssp* by 50%. Clindomycin was sensitive to *pseudomonas ssp* 33% while *pseudomonas ssp* was resistant to clindomycin 67%. *Pseudomonas ssp* was resistant to Erythromycin 100%.

Concerning for sheep the figure 3 shows that, vancomycin was sensitive to *Pseudomonas* ssp with 75% and *Pseudomonas* ssp was resistant to vancomycin with 25%. Ciproflaxacin was sensitive to *pseudomonas ssp* 62.5% and *pseudomonas* ssp was resistance ciproflaxacin with 37.7%. Cefuroxime was sensitive to *pseudomonas ssp* 87.5% and *pseudomonas ssp* was resistant to Cefuroxime 12.5%. *Pseudomonas ssp* was resistant to Nitrofuranton 25% and Nitrofuranton was sensitive to *pseudomonas ssp* by 75%. Clindomycin was sensitive to *pseudomonas ssp* 12.5% while *pseudomonas ssp* was resistant to clindomycin 87.5%. *Pseudomonas ssp* was

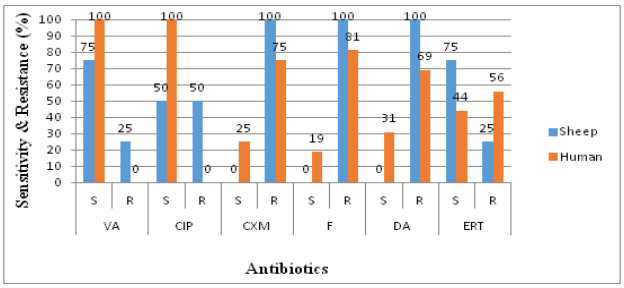

Figure4. *Resistance of human and sheep Staphylococcus aureus to antibiotics*

The results from figure 4 show that for human; vancomycin and ciproflaxacin were sensitive to Staphylococcus aureus 100%. Cefuroxime was sensitive to Staphylococcus aureus 25% and Staphylococcus aureus was resistant to Cefuroxime 75%. Nitrofuranton was sensitive to Staphylococcus aureus 19% while Staphylococcus aureus was resistant to Nitrofuranton on 81%. Clindomycin was sensitive to Staphylococcus aureus 31% while Staphylococcus aureus was resistant to clindomycin 69%. Erythromycin was sensitive to Staphylococcus aureus 44% and Staphylococcus aureus was resistant to Erythromycin 56%.

In sheep, figure shows that vancomycin was sensitive to staphylococcus aureus with 75% and staphylococcus aureus was resistant with 25%. Ciproflaxacin was sensitive to Staphylococcus aureus 50% and Staphylococcus aureus was resistant with 50%. Staphylococcus aureus was resistant to Cefuroxime 100%. Staphylococcus aureus was resistant to Nitrofuranton on 100%. Staphylococcus aureus was resistant to clindomycin 100%. Erythromycin was sensitive to Staphylococcus aureus with 75% and Staphylococcus aureus was resistant to Erythromycin with 25%.

4. DISCUSSION

4.1. Prevalence of Bacteria Crossed from Sheep to Human

The frequency each common isolated bacterium has meaning due to how they breed. E. Coli is mostly found in guts of cow especially in calves, sheep, goat, and deer. These are main animals that transmit E. coli to human including sheep that this study focused on. People can be contaminated through undercooked ground beef, raw milk, but on this study, it is simple that families residing with sheep

get contamination through the direct contact with stools and if one person in the family get contaminated it facilitates the spread of infection between family members. Thus the frequency of E. coli here is high due to that sheep is among main mammals that hosts E. coli and no doubt if stays with human, it will transmit E. coli hosted. In this study, Escherichia coli was isolated within sheep and human living together on 43.3% and 26.6% respectively (Figure 2). The high frequency of E. coli is an evidence of transmission of this bacteria to human. This study is in the same agreement with a study conducted by Chapman et al. (1997) where 25.8% of E. coli was isolates of cultured samples of lambs living with human. These results are not far from a study conducted in Ethiopia by Kibret & Abera (2014) showed that E. coli was predominating isolate with 63.6%. S. aureus was screened with 20.8%. E. coli was 20.8% among all sheep studied in Giza, Ethiopia (19).

Coming to Pseudomonas ssp, you better know that it has various hosts. Coming to their breeding sites, commonly Pseudomonas bacteria breeding sites are water, soil, and damp. Sheep is one of animals that mostly exposed to the breeding site of this bacteria, they are herbivorous where they can get contamination from their herbal food, in their shelter, mostly always wet due urination which favor this bacterium to multiply, thus, if they stay with mankind it is easy to transmit to human, and surely that's the prevalence of Pseudomonas ssp has been high in sheep comparing to human.

In the present study, pseudomonas was isolated with 26.6% in sheep and 10% in human. Pseudomonas ssp were isolated with 43.3 and 26.6 % respectively (Figure 3). Among human, Escherichia coli and Pseudomonas ssp were isolated with 26.6 and 10 % respectively.

Staphylococcus aureus is the microflora of humans. It grows between 4-460c and human temperature range included and that's why it mostly fond in human skin. It is also found in some food that human consume like salad, cream-filled pastries and others. At this case a human can firstly transmit E. coli to sheep according to the study, that is why you find high frequency of this bacteria in human regarding the above figure. Staphylococcus aureus was the only gram positive bacteria isolated for both human and sheep with 23.3 and 13.3% respectively (figure 2). Microbiological analysis revealed that 27 (36.5%) out of 74 sheep were infected with Staphylococcus spp20. The study conducted on clinical examination, the incidence of pseudomonas spp. was found to be 5.88% sheep and 7% human respectively (21).

There was an evidence association of human bacteria resulting from sheep ($P>0.05$, $P=0.003$). This is an indicator showing that some of human diseases can be transmitted by domestic animals.

4.2. Isolated Bacteria and Their Responses to Antibiotics

In this study antibiotics had high sensitivity to E. coli, Pseudomonas ssp and S. aureus as vancomycin was sensitive to all bacteria 100%. By comparing to other study, 25.8% of isolated E. coli was resistant to four different antibiotics (22).

In the present study, Pseudomonas ssp was also sensitive 100% to Erythromycin. By comparing to the study conducted in Ethiopia showed that the overall resistance rate of Pseudomonas ssp to erythromycin was 85.6% (23). In Giza, Ethiopia, isolates bacteria were 100% sensitive to Ciprofloxacin.

resistant to Erythromycin 100%. The same way to E. coli, Pseudomonas ssp has been shown be more prevalent in sheep, which show that is fevered the. Antibiotic resistance has been shown to be high in sheep, mostly when animals are treated for any infection, the chance to take it far from infection source seem to be low, which a factor leading to antibiotic resistance in animals including sheep. We early saw that pseudomonas' breeding sites are all expose the sheep and animals and without doubt, this influence the antibiotic resistance and surely that is why antibiotic resistance has been high in sheep compared to human.

In the present study vancomycin was sensitive to staphylococcus aureus with 75% and staphylococcus aureus isolated in sheep was resistant with 25%.

Looking at statistics above, the E. coli isolated from sheep is more resistant to that of human, and this means something important to consider. Some factors leading to antibiotic resistance including poor hygiene, overuse and underuse of antibiotics, mutation of bacteria, and others. The living conditions that sheep lives favor bacteria growth comparing to the sheep itself which increase antibiotic resistance in sheep compared to human. Antibiotic use in agriculture increase also antibiotic resistance in animals including sheep, as interpreted early, this bacterium is favored to grow in

sheep's environment as well as other animals and sometimes animals' health is taken in consideration only if they sick which really increase the chance of the bacteria to resist the effect of antibiotics. The burden of sheep for this bacteria is not like that of human as mentioned earlier which also show inequality in antibiotic resistance.

The difference is hire compared to previous two bacteria. For Staphylococcus aureus, we have seen that is favored by human condition, but now sensitivity for human is high compared to that of sheep. This can happen and is very understood, even if human harbor this bacteria, but human health is more taken in consideration compared that of sheep which can influence of low resistance compared to sheep. Mutation of bacteria cannot be a fact to reject, this bacterium as well as others can do mutation from one environment to other, it can also happen to Staphylococcus aureus once transferred from human to sheep or from sheep to human. Mostly human is treated while animals wait sometimes an outcome to treat itself, this increase their immunity to resist on infection but also increase the antibiotic resistance, this can be the case on Staphylococcus aureus.

The other study conducted in Gaza showed that staphylococcus isolates from sheep were 100% sensitive to Ciprofloxacin and Vancomycin (19). More specifically 52.5% of bacteria isolated from both sheep and sheep keepers were resistant to tetracyclines, 41.2% to ampicillin, 29.8% to spectinomycin, 23.7% to amoxicillin clavulanate, 13.9% to trimethoprim sulphonamide and 11.2% to neomycin (21).

5. Conclusion and Recommendation

5.1. Conclusion

This study was conducted to study common shared bacteria and their antibiogram between human and sheep. Urine specimens were collected for both human and sheep. Culture, gram stains, biochemical test and antibiogram test were performed. Isolated bacteria were E. coli, Pseudomonas ssp and S. aureus. Bacteria were more isolated in sheep than in human. Antibiotics were more susceptible in bacteria isolated from sheep than those isolated from human. According to the results of this study, bacteria are isolated from human and sheep. Sheep have high number of bacteria than human. Therefore, animals could not live with human as it can cause zoonotic infections that are deadly fatal.

5.2. Recommendation

Based to the results of this study, many researches must be conducted in different part of country on all domestic animals. People could live differently with animals. Government could implement polices of helping people to gain facility of animal farming in good condition. To implement policy of education people about the effect of living with animals.

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