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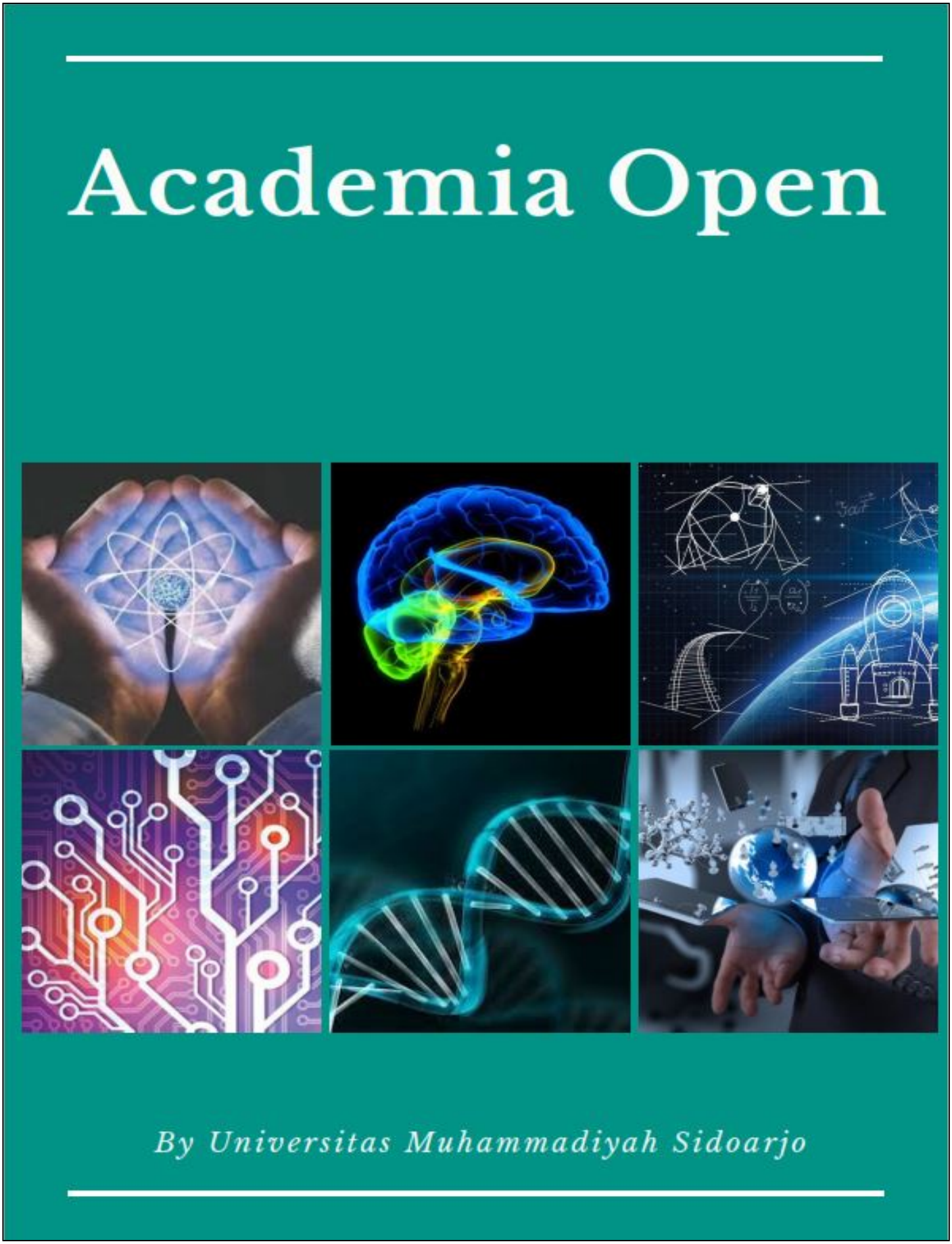

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Assessing Zoonotic Bacterial Pathogens: Risks and Public Health Implications from Livestock in Residential Areas of Mosul

Menilai Patogen Bakteri Zoonosis: Risiko dan Implikasi Kesehatan Masyarakat dari Ternak di Daerah Pemukiman di Mosul

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Abstract

Despite the increasing frequency of cattle in residential areas, particularly in residential neighbourhoods, little is known about the potential health hazards associated with their presence. This cross-sectional investigation examined the incidence of zoonotic agents in 108 cattle samples collected from various locations around Mosul city. The standardized medical examination and livestock owner interviews occurred from October 4 to December 18, 2024. We identified bacterial pathogens in samples from each animal's pharynx, nose, ear, mouth, and faeces. We determined that all of the cattle were in excellent health. Out of 108 cattle, 84 (or 78% of the total) tested positive for zoonotic agents. The principal pathogen was ESBL Escherichia coli (E. coli) isolated from 51 (60.7%) faecal samples. We isolated extended-spectrum beta-lactamase C. difficil organisms from 16 cows (19%). We isolated MRSA from 12 (14.3%), VRE from 3 (3.6%), and Salmonella spp. from 2 (2.4%) of the cows. ESBL E. coli showed significant resistance, particularly to amoxicillin (86.3%) and gentamicin (78.4%). Clostridium difficile exhibited complete resistance to amoxicillin (100%), while MRSA demonstrated full resistance to several antibiotics, including gentamicin and vancomycin. VRE and Salmonella spp. also displayed high resistance rates.

Highlights:

1. Zoonotic Agents in Cattle: 78% tested positive in residential areas.
2. Pathogen Resistance: ESBL E. coli, MRSA, and others showed high resistance.
3. Health Hazards: Antibiotic-resistant bacteria pose significant public health risks.

Keywords: Zoonotic, bacterial pathogens, public health, cattle, multi-drug resistant

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Introduction

The increasing proximity of livestock to human residential areas has raised significant concerns regarding the potential transmission of zoonotic diseases, particularly bacterial pathogens, that pose serious public health risks. In many developing regions, such as the outskirts of Mosul, Iraq, cattle and other livestock are commonly found roaming or being raised in close contact with human dwellings, elevating the risk of zoonotic transmission. Animals can transfer zoonotic diseases to humans through direct contact, indirect contact via contaminated surfaces, or consuming contaminated animal products. Numerous bacterial pathogens, such as Salmonella, Brucella, E. coli, and Campylobacter, cause numerous infections, including gastrointestinal infections and life-threatening ones like sepsis and meningitis in cattle [1]. These germs frequently infect stock, especially cows, and when animals roam freely, the likelihood of their spread increases because they may contaminate water, soil, and even food [2].

A recent study [3] revealed that livestock-caused zoonoses constitute a significant portion of the emerging infectious disease. Urban and peri-urban areas, such as Mosul, where human and livestock contact is common, exacerbate this issue. In these places, the absence of proper veterinary care, poor animal welfare, and cleanliness further heighten the risk factors of humans getting an infection from the animals. Intensive farming and inadequate waste management systems contribute to water, soil, and air contamination with zoonoses, which these vehicles can easily transmit [4-6]. Additionally, the environmental pathogen Bacillus anthracis, which causes anthrax, is common in cattle farms, especially where infection control measures are not strong enough [7-8]. This means that Coxiella burnetii, which causes Q fever, could be passed from animals to people without barriers.

Considering the example of Mosul, the post-conflict period and ongoing socio-political turmoil have posed significant challenges to the sustainability of healthcare systems, including veterinary and animal health services. Further consideration of the ear inside the ear leads to a steady increase in risk factors for outbreaks of zoonotic diseases, as people and various animals typically compete for the same water and food supplies [9]. Inadequate public health awareness about the threat of zoonotic diseases further aggravates these problems. People are unaware of the risks of allowing free-range domestic animals within the compounds. Therefore, there is a significant knowledge gap in conducting studies about the prevalence of zoonotic bacterial pathogens in cattle and their potential risk to the health of people in Mosul and related areas [10].

Earlier studies have confirmed that, to a great extent, cattle are a reservoir for bacteria of zoonotic origin. For instance, Brucella abortus and Brucella melitensis are typically present in cattle. They are known to cause a human body disease called brucellosis, which involves the symptoms of fever, feelings of discomfort, and chronic pain of the joints [11]. Furthermore, consuming uncooked meat or contaminated dairy products can transmit Salmonella enterica, a common pathogen in bovine animals, to humans, causing gastroenteritis [12]. Besides, E. coli O157, an opportunistic pathogen attached to cattle, has been reported to cause diarrhoea and haemolytic uremic syndrome HUS in normally healthy people, especially those most at risk, like children and older persons [13–15]. These pathogens are highly found in animals and their faecal products. They can eventually drain water sources, soils, and crops, thus seriously threatening public health, especially to people living near cattle [16].

Recent concerns highlight a high burden of bacterial zoonoses in regions heavily populated by cattle, especially where human and animal habitats are poorly segregated. To illustrate, an inquiry by [17] noted that the rural zones of Southeast Asia, where livestock can readily enter households, have a high rate of zoonosis infection compared to metropolitan areas where livestock are confined and management practices are followed. This situation is also seen in parts of Mosul where cattle rounded up with people are common, especially in the outskirts of the city where there is no proper control of animals and veterinary health practitioners [18]. These conditions enable quick movement and spread of pathogens, especially in environments with poor sanitation standards and where there are few or no medical facilities that can handle a public health emergency concerning the outbreak of infectious diseases [19].

The impact of zoonoses as a category of diseases on population health is a growing concern in the field of public health. This is due to the increased focus on new infections like COVID-19, which has highlighted the need for a deeper understanding of the dynamics of zoonotic infection transmission [20-21]. Zoonotic bacterial pathogens of animals, particularly cattle, pose a potential persistent problem in communities where livestock rearing is the people's livelihood and are kept in close quarters with humans. The issue is particularly challenging in Mosul due to the fragmented healthcare and veterinary services, which are still recovering from the effects of years of conflict [22]. These factors highlight the need for HW approaches such as the One Health framework that has advocated for a concerted effort by several sectors at all levels locally, regionally, and worldwide to address health issues of people, animals, and the environment [23–24].

Large herds of cattle act as a reservoir for zoonotic bacterial pathogens. Yet, the public health aspects of these diseases remain largely unexplored in Iraq, particularly in the city of Mosul. Available studies have also pointed to zoonotic disease underreporting, associated with a lack of proper surveillance and services, as part of the barriers to diagnosing and managing such infections by the local healthcare systems [25-26]. It is critical that these animals are appropriately tested and that the risks to public health are determined. This can be aided by carrying out studies bearing in mind the local livestock population and the potential zoonotic pathogens. Results obtained from such studies may be used in the design of strategies to prevent the emergence and spread of zoonotic disease

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among populations surrounding animals [27].

Given these concerns, this study aims to assess the public health risks posed by common zoonotic bacterial pathogens in cattle populations in the Mosul district and emerging risks. A baseline data set on the burden and distribution of zoonotic bacteria within the local cattle population will be created by identifying and isolating bacterial pathogens from cattle samples using microbiological and molecular methods. The study will also identify the risk factors that aid the transmission of these agents to propose measures that can be adopted at the community level to prevent infections.

Methods

2.1 Study Area and Sampling

This study was conducted in four different and similar rural settings regarding logistics and accessibility within the Mosul city limits (Baaj, Sinjar, Hammam al-Alil, and Zumar located towards north-west Mosul).

The sample size of 108 cow specimens was considered necessary to obtain a certain minimum prevalence equal to or higher than 7% within a margin of error of 0.10 at the 95% confidence level. Since no published data on the prevalence of certain cattle diseases in Mosul, this value was arrived at using scholarly works done in Iraq [28–30].

The cattle were assessed from October 4 to December 18, 2024. Swab samples were collected from the ear, nose, mouth, and throat and placed in a sterile transport medium. To activate, two swabs were obtained from each sample, which were then promptly immersed in thioglycolate broth. The faecal sample was immersed in a tetrathionate broth. Subsequently, each swab was placed separately in an aerobic and anaerobic medium.

A cooler was utilised to contain all samples throughout their transportation to the specialist veterinary laboratory in the city heart of Mosul. Furthermore, physical examinations were conducted with a predefined checklist to evaluate the general health status of each animal involved in the study.

Owners were questioned via a standardised, pre-tested questionnaire to get a comprehensive history of each cattle. Enquiries encompassed specifics of the animal's dietary habits, medical history, current or prior treatments, and living environment.

2.2 Bacterial Identification

Salmonella spp. was isolated by plating one loop of defrosted excrement directly onto XLT and Brilliant Green agar (Oxoid) [31]. Afterwards, the plates were maintained at 37.8 °C for a half-day. To provide variety, faeces samples were incubated in Mueller-Kaufman tetrathionate broth (Oxoid) for 48 hours at 37 °C before being distributed over XLT and Brilliant Green agars. Colonies that shared a Gramme stain morphology were tested by IMViC test, API20 E test, and latex agglutination (Oxoid) were identified as Salmonella spp. [32].

Rectal swabs and excrement were treated with an enrichment technique to isolate C. difficile. Eight millilitres of CCF soup containing 0.1% sodium taurocholate was mixed with swabs contaminated with faeces. We maintained the tubes at 37.8C in an aerobic environment for seven days. The next step was to transfer 2 mL of soup from each tube to a clean tube, combine it with the same volume of 100% alcohol, and let it sit for 30 minutes at room temperature [33]. Ten minutes were spent spinning them at 8000 g. After discarding the liquid portion, the pellet was dispersed on blood agar and cultivated in an oxygen-free environment set at 37 °C for 48 hours. The same approach was used to check negative samples after 14 days in an anaerobic chamber. The distinctive odour and form of C. difficile colonies and their colour changes on CCF agar plates, Gramme stain, and l-proline-aminopeptidase synthesis allowed us to identify them [34].

The methicillin-resistant Staphylococcus aureus (MRSA) bacteria were cultivated in a broth containing tryptone, mannitol, yeast extract, and 7.5% sodium chloride for 24 hours after being immediately added to mannitol salt agar with 2 mg/mL oxacillin. Over 48 hours, the inoculation plates were maintained at 35°C in a 5% CO2 environment. In order to identify the MRSA isolates, a combination of a positive coagulase reaction and a positive latex agglutination test for S. aureus were employed. This analysis used the Passtorex Staph-Plus kit, which Bio-Rad manufactured in Hercules, CA, USA. The bacteria were confirmed to be methicillin-resistant by using a latex agglutination test and observing growth on Mueller-Hinton agar supplemented with 6 mg/mL oxacillin (Oxoid) [35].

Using both direct and enrichment approaches, we followed Oxoid's directions to grow faeces samples for VRE. This business created the cultural media. According to the standards by [36], the API Strep biochemical identification test (Oxoid) confirmed that the catalase-negative and Gramme-positive cocci were enterococci. Researchers followed international recommendations to evaluate the lowest inhibitory dosage for vancomycin resistance and employed E-test strips (BD Biodisk, Solna, Sweden).

After inoculating EMB and MacConkey agar with 2 mg/mL of cefpodoxime (Oxoid) and maintaining the culture at 37 °C for 48 hours, we were able to cultivate ESBL E. coli bacteria from faeces. The IMViC, API20 E tests, and a

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small approach for biochemical testing were subsequently carried out [37–38].

2.3 Antibiotic Susceptibility test

We conducted the antibiotic susceptibility test for E. coli, C. difficile, MRSA, VRE (Vancomycin-resistant Enterococcus), and Salmonella spp. using the Kirby-Bauer disc diffusion method, standardised to a 0.5 McFarland turbidity standard [39-40]. We cultured the bacterial isolates on appropriate growth media and then incubated them. To make a 0.5 McFarland suspension, the bacterial cultures were diluted in saline until the cell density was 1.5 x 10^8 CFU/mL. We uniformly swabbed the suspension onto the surface of Mueller-Hinton agar plates (or any other suitable agar depending on the organism, such as Brucella agar with supplements for Clostridium dWe then placed antibiotic-impregnated discs on the agar surface using sterile forceps. e forceps. The antibiotics used were Amoxicillin (16 µg), Ciprofloxacin (5 µg), Gentamicin (30 µg), Doxycycline (30 µg), Vancomycin (30 µg), Erythromycin (5 µg), Ceftiofur (30 µg), Cefotaxime (30 µg), Trimethoprim-Sulfamethoxazole (25 µg), Metronidazole (5 µg), and Oxytetracycline (30 µg). These antibiotics were chosen based on how well they worked iWe incubated the plates at 35°C for 18–24 hours. We measured the inhibition zones around the antibiotic discs in millimetres after incubation. llimetres. We determined the susceptibility by comparing the zone sizes to the standardised breakpoints defined by the Clinical and Laboratory Standards Institute (CLS) [41]. Based on these breakpoints, we recorded the results as susceptible, intermediate, or resistant. The test provided a profile of each organism's resistance or susceptibility to the antibiotics tested, which was crucial for guiding effective treatment strategies.

Result and Discussion

Result

Only 108 of the 122 cows that volunteered for the study could fulfil the participation requirements. The average

age of the cows was 7.22 years, with a standard deviation of 4.7 years, and the median age was 7.5.

The lifespan of the cows varied from one to sixteen years. A single cow that was one year old was the only one present. Every single cow was subjected to a comprehensive collection of samples. Table (1) summarises the findings from several laboratory tests,

including culture, faecal flotation, and others. Among the positive isolates, E. coli was the organism that was found the most frequently, accounting for 51 (60.7%) of the total; this was followed by C. difficile, which was found in 16 (19%), MRSA, which was found in 12 (14.3%), VRE, which was found in 3 (3.6%), and Salmonella spp., which was found in 2 (2.4%). The results of antibiotic susceptibility testing indicated that different bacteria exhibited varying degrees of antibiotic resistance. Amoxicillin was resistant to 86.3% of the ESBL E. coli isolates (51 total), ciprofloxacin was resistant to 31.4%, gentamicin was resistant to 78.4%, and doxycycline was resistant to 60.8%.

Bacterial Pathogen	Specimen source	Specimens number n= 84
ESBL E. coli	Faeces	51 (60.7%)
Clostridium difficile	Faeces	16 (19%)
MRSA	nasal swab	12 (14.3%)
VRE	Faeces	3 (3.6%)
Salmonella spp	Faeces	2 (2.4%)

Table 1. *Quantity of isolates of possible animal diseases in a livestock sample of cattle from the suburbs of Mosul city*

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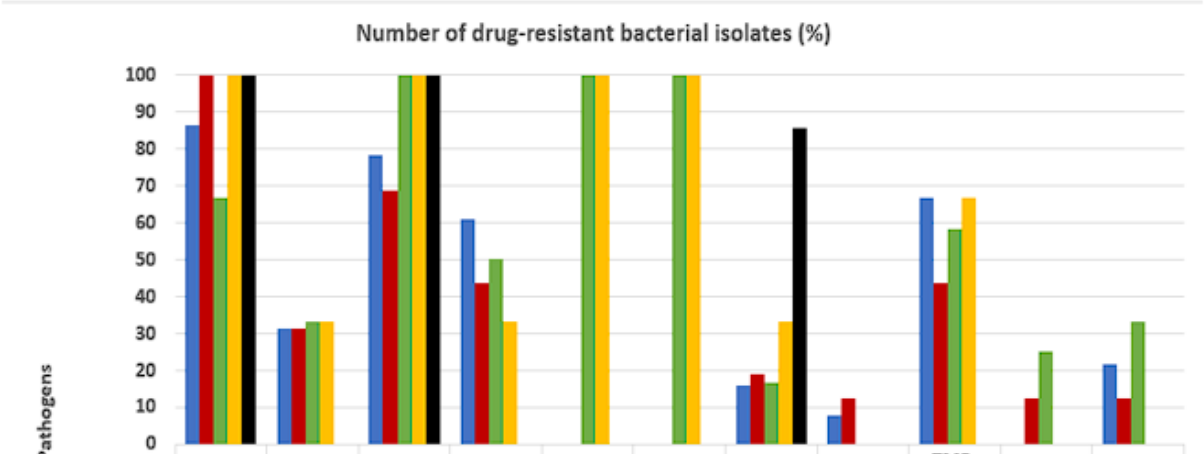

Pathogens	AMX	CIP	GEN	DOX	V	E	CTX	CEF	TMP-SMX	MEZ	OXY
E. coli	86.3	31.4	78.4	60.8			15.7	7.8	66.7		21.6
C. difficile	100	31.3	68.8	43.8			18.8	12.5	43.8	12.5	12.5
MRSA	66.7	33.3	100	50	100	100	16.7		58.3	25	33.3
VRE	100	33.3	100	33.3	100	100	33.3		66.7	0	0
Salmonella spp.	100	0	100	0			85.7	0	0		

Figure 1. *Antibiotic Susceptibility Testing Outcomes for Bacterial Isolates from Cattle Samples Collected in Residential Areas of Mosul. AMX; Amoxicillin, CIP; Ciprofloxacin, GEN; Gentamicin, DOX; Doxycycline, VAN; Vancomycin, E; Erythromycin, CTX; Cefotaxime, CFE; Ceftiofur, TMP-SMX; Trimethoprim-Sulfamethoxazole, MEZ; Metronidazole, OXY; Oxytetracycline.*

Resistance to cefotaxime was 7.8%, whereas resistance to erythromycin was 15.7%. To put it into perspective, 66.7% of the isolates resisted ceftiofur, whereas 21.6% resisted trimethoprim-sulfamethoxazole. Two hundred and 8% of the sixteen isolates of C. difficile were resistant to amoxicillin. Additionally, 68.8% of the isolates were resistant to gentamicin, 43.8% to doxycycline and ceftiofur, 18.8% to erythromycin, and 12.5% to cefotaxime and trimethoprim-sulfamethoxazole simultaneously. In a total of twelve isolates, MRSA demonstrated a 100% resistance to gentamicin, doxycycline, vancomycin, and cefotaxime; 66.7% resistance to amoxicillin; 33.3% resistance to ciprofloxacin and trimethoprim-sulfamethoxazole; and 58.3% resistance to ceftiofur. Three VRE isolates showed complete resistance to all antibiotics: amoxicillin, gentamicin, doxycycline, vancomycin, and cefotaxime. The age of patients resistant to ceftiofur was 66.7%, whereas 33.3% were resistant to ciprofloxacin and trimethoprim-sulfamethoxazole. Two isolates of Salmonella spp. demonstrated full resistance to amoxicillin and gentamicin (100%), resistance to oxytetracycline (85.71%), and no resistance to ciprofloxacin or doxycycline (no resistance is found) (table 2).

Discussion

The present study, which focused on zoonotic bacterial pathogens from livestock in residential areas of Mosul, underscores significant public health concerns arising from antibiotic-resistant bacteria in the environment. Zoonotic agents, which are transmitted from animals to humans, pose a substantial threat, particularly when they exhibit multidrug resistance (MDR). The study involved 108 cattle, with bacterial pathogens isolated from 84 animals (78%). Among these, ESBL E. coli was the most prevalent pathogen, isolated from 51 (60.7%) cattle. This high prevalence of ESBL E. coli is alarming because extended-spectrum beta-lactamase (ESBL)-producing bacteria are known to resist a wide range of beta-lactam antibiotics, rendering many common treatments ineffective. The study also identified C. difficile in 16 cattle (19%), MRSA (Methicillin-resistant Staphylococcus aureus) in 12 (14.3%), VRE in 3 (3.6%), and Salmonella spp. in 2 (2.4%) of the cattle. The antibiotic susceptibility testing revealed concerning levels of resistance, particularly in E. coli and MRSA isolates, which demonstrated significant resistance to key antibiotics such as amoxicillin, gentamicin, and vancomycin.

The reason for the high rates of ESBL E. coli in cattle would therefore be in agreement with findings from studies in regions where there are high livestock loads and little infection control. For instance, the ESBL E. coli isolation rate was also high, especially in rural areas where farming practices are quite common, according to [42]. The patterns of resistance seen in this study also follow the trends mostly observed elsewhere concerning ESBL strains, which are known to be highly amoxicillin and gentamic resistant [43]. The rates of amoxicillin (86.3%) and of gentamicin in our study (78.4%), which is the third antimicrobial administered and which targets Gramme

negatives, are within the range of results presented by [44] on livestock studies from Japan. The high-level resistance to these antibiotics indicated that there is a possibility that excessive or inappropriate use of these drugs in animals, if any, is driving the emergence and spread of resistant strains.

Additionally, the isolation of C. difficile in 19% of the cattle, especially its 100% resistance to amoxicillin, is consistent with European studies where C. difficile has become the key zoonotic pathogen affecting livestock [45]. This concern is heightened when this pathogen, which was found in beef cattle, can be transmitted to humans, resulting in severe gastrointestinal diseases. C. difficile susceptibility toward gentamicin, which is 68.8%, and doxycycline, which is 43.8%, also adds barriers toward successful clinical management of the disease as these drugs have important applications in human and veterinary medicine.

Out of the 14.3% of cattle from which MRSA was isolated', it was observed that 'MRSA was resistant to all of the antibiotics tested, including gentamicin, doxycycline, vancomycin, and ceftazidime, with 100 percent resistance patterns'. Higher levels of this resistance have also been noted in other studies, for example [46], who also reported high resistance rates in MRSA strains associated with livestock in Europe. The implication for the health of humans from the emergence of MRSA in livestock is high, more so for those in direct contact with animals, such as farmers and veterinary officers. MRSA resistance to vancomycin, a reserve drug for severe bacterial infections, is of particular interest and concern. When other first-line drugs fail, this offers few options for treatment.

Although less common in our study (3.6% of cattle), VRE was very interesting since it was resistant to all tested antibiotics, including vancomycin. Of note, the detection of VRE at the level of livestock deserves attention because VRE diseases in humans are hard to manage and have high risks of death [47]. These findings are similar to those of [48], who observed VRE in 5% of the cattle population in the course of a large-scale European study. The finding of VRE in cattle in Mosul shows that this organism can spread out from the hospitals, be transmitted through the food chain, and pose a danger to the community.

The isolation of Salmonella spp. from 2.4% of the cattle and its 100% resistance to amoxicillin and gentamicin is consistent with other studies conducted in the region. As noted in a study `by [49]: Antibiotic overuse contributes to a high percentage of resistant Salmonella isolates in livestock. This in vivo resistance of Salmonella to oxytetracycline observed in this study was quite high (85.71%), and this presents another problem in the management of the infection since tetracyclines, which include oxytetracycline, are mainly used in treating animals. The good news is that there have been no reports on the resistant rate of ciprofloxacin and doxycycline as they apply to this region, meaning there may still be potential cures for Salmonella infection.

This study's findings are consistent with the global patterns over the emergence of antibiotic-resistant zoonotic pathogens in farm animals. The prevalence of ESBL E. coli in cattle was 70.7%, which is also reported in studies in other countries, such as those by [50], who reported 58% in cattle in Bangladesh. Furthermore, the high levels of resistance to amoxicillin and gentamicin from our data were also similar to some European and Asian studies where these antibiotics are commonly employed in livestock production [51]. The efficacy of the antibiotic E. coli ESBL isolates in this study calls for improved regulation of antibiotic usage in animals to curb resistance strains.

D. difficile was isolated from only 19% of the cattle in this study, which is higher than the prevalence recorded in other studies. For example, Knight and colleagues reported a C. difficile infection prevalence of 12% of cattle out of those examined in the United Kingdom [52]. The higher prevalence in the study points to factors that may include the environmental conditions or types of farming carried out in Mosul. However, the resistance rates to amoxicillin and gentamicin or doxycycline were as seen in other studies, with C. difficile showing high amoxicillin resistance and moderate to low gentamicin and doxycycline resistance [45]. While these findings demonstrate a correlation between clostridium associative bacteria and livestock, more extensive studies of C. difficile in livestock will be required to assess its epidemiology and resistance mechanisms.

The prevalence observed in this study (14.3%) for MRSA is lower than elsewhere. For example, similar to our study, Palaga et al. (PA25) found MRSA in livestock of the United Kingdom at a 20% level [53]. The majority of resistance features of our study remained along those lines, especially the most 100% resistant to patients, gentamicin and vancomycin, which were reported elsewhere. The dispersal of livestock and the strains of MRSA associated with animals has contributed to the emergence of multidrug-abounding isolates of more infections [46].

In our study, only 3.6% of the surveyed cattle were found to carry VRE, and this is in agreement with the results of similar studies conducted in Europe and North America. For instance, a survey performed by [54] documented the existence of VRE in 4% of livestock in the United States. In this study, VRE strains resistant to all antibiotics are included, where all VRE strains tested were susceptible to all antibiotics at another study [55], which mirrors our findings. VRE identification among livestock suggests that more comprehensive antibiotic stewardship programs are necessary to help curb resistance as these organisms are highly transmissible.

The prevalence of Salmonella spp. In our study (2.4%) is not as high as that reported in other studies. For instance, [56] reported the occurrence of Salmonella in 6% of cattle in Kenya. Although some of them were not upon collection, patterns of resistance observed in this study, such as 100% amoxicillin and gentamicin resistance, are similar to findings from other studies on RTS patients. It is a good sign in our study that there was no resistance to ciprofloxacin and doxycycline because the antibiotics are often employed in the management of Salmonella

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infections in humans as well as animals [57].

Concluison

According to the paper, zoonotic bacterial pathogens in livestock in urban Mosul are a cause for concern in terms of public health. Showing point wise understanding of the abstract, our research UCSD reveals that antibiotic resistant strain especially ESBL E. coli, C. difficile, MRSA, VRE, Salmonella spp. showed a very high prevalence with alarming levels of multi-drug resistance to common agents such as amoxicillin, gentamicin and vancomycin. This supports possible health claims of simplified infection prevention and control and appropriate antibiotic use in livestock to curb the emergence of resistant zoonoses. However, within the bounds of this study, we conclude, that perhaps the position of antibiotics referred to in livestock growing norms further develops in all countries of the world: this means that veterinarians, public health officials and policy makers are supposed to work simultaneously in resolving the issues. Active monitoring, compliance with sanitary norms and expedient purposive restriction of the use of antibiotics will be necessary for the prevention of the transfer of drug-resistant strains from animals to humans, and their carrying out in urban or rural urbanised zones.

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