Prevalence and susceptibility profiles of *Campylobacter jejuni*, *Escherichia coli* and Salmonella in dairy farms in Mukono District, Uganda

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Prevalence & susceptibility profiles of *Campylobacter jejuni*, *E. coli* & Salmonella in dairy farms in Mukono District, Uganda

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Presentation Outline

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Introduction

- Foodborne diseases are a challenge for both developed and developing countries (Da Cuhna *et al*., 2012), are a leading cause of illness and death in developing countries (Hassan *et al*., 2010).
- *E. coli*, Salmonella & Campylobacter species are among the commonest causes of GIT diseases in SSA (Fletcher *et al*., 2011).
- Dairy cattle are common carriers of *foodborne pathogens* (Stanley & Jones, 2003, Kaakoush *et al*., 2015).
- *Campylobacter* infections are frequently acquired from foods of animal origin (Hakkinen *et al*., 2007).
- Rising microbial infections prevalence is increasing use of antimicrobial agents for animal health in addition to use of large quantities for growth promotion & prophylaxis in food animals (Hakkinen *et al*., 2007; CDC, 2013) thus accelerating emergence of AMR.

Introduction

- Monitoring prevalence & susceptibility profiles of food borne pathogens such as *Salmonella*, *E.coli* & *C. jejuni* is necessary given their public health implications (Hur *et al*., 2012).
- Developed countries have antibiotic resistance monitoring programs e.g. USA (NARMS), Japan (JVARM), Canada (CIPARS), Sweden (SVARM), Netherlands (MARAN), Denmark (DAN-MAP) and Norway (NORM-VET).
- Developing countries like Uganda lack established AMR monitoring programs (Knezevic and Petrovic, 2008).
- The study assessed prevalence and susceptibility profiles of *C. jejuni*, *E. coli* & Salmonella.

Materials & Methods

- The study was conducted in Mukono district, an important dairy producing area. Repeated cross-sectional studies were conducted in one year from June 2018 to May 2019. Proportional sampling was used to select the study farms from the nine parishes.
- Samples were collected from selected dairy farms in nine parishes (Bulijjo, Ddundu, Kabembe, Kiyonga, kyampisi, Nanga, Namengo and Namuyenje from three Sub-counties (Kimenyede, Kyampisi & Nakisunga), Mukono district.
- 184 samples were collected comprising milk (57), fecal (65) & environmental (62).
- 60 ml pooled milk sample was aseptically collected from four quarters - udder of each lactating cow into 100 ml sterile sample collection bottle.
- Fecal & environmental samples were aseptically collected into sterile 50 ml stool collection bottles and 500 ml Ziploc bags. All samples were transported in a temperature monitored cooler box to the Microbiology Laboratory at COVAB, Mak University within 4 hours & immediately processed.

Visits to the study farms during sample collection

Visits to the study farms during sample collection cont'd

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Materials & Methods

- Samples were received in the lab, recorded & given lab identification codes before processing.
- Sample inoculation for bacterial isolation was by the agar surface streaking method & broth dilution.
- Controls used were *C. jejuni* ATCC 29428, *E.coli* ATCC 25922 & Salmonella ATCC 13076.
- *E. coli* was isolated on Chromogenic Coliform agar (Oxoid, UK) at a temperature of 37°C, 24h, forming characteristic dark blue colonies. Other coliforms were pink on the same medium.
- Confirmatory identification was by biochemical tests IMViC (Indole, Methyl red, Voges Proskauer & Citrate utilization test).
- *E. coli* isolates were Indole & Methyl red positive, but VP & Citrate negative. *E. coli* isolates were tested for AS by the single disc diffusion method against eight standard antibiotics; Gentamycin, Ciprofloxacin, Ampicillin, Oxacillin, Cefoxitine, Cefotaxime, Ceftazidine and Ceftriaxone.
- AST results were interpreted & reported as sensitive (S), intermediate (I) or resistant (R) according to the CLSI / NCCLS (2005) interpretive chart.

Summary of detection & confirmation methods used in the study: Materials & Methods

Target Bacteria	Method	Confirmation
E. coli	Agar streaking (Quadrant)	Indole, Voges Proskauer, Methyl red & Citrate utilization
Salmonella	Pre-enrichment, enrichment & Surface streaking	TSI, Urease production & Citrate utilization
Campylobacter jejuni	Pre-enrichment, enrichment & surface streaking	Gram staining, Oxidase test, Motility testing & Hippurate hydrolysis

Results

- Out of 184 samples collected from 33 study farms, *E.coli* was isolated in 40 (21.7%) samples.
- *Campylobacter jejuni* & Salmonella were not isolated in all the samples tested.
- *E.coli* was isolated in 56.9% (37/65) of fecal and 4.8% (3/62) of water samples.
- *E.coli* was not isolated in all 57 milk samples tested.
- Out of the 33 study farms, 16 (48.5%) had *E.coli* isolated from fecal samples.
- One farm had *E.coli* isolated from both the fecal and water samples.

AST for bacterial isolates against different antibiotic discs.

- *E.coli* isolates were tested for susceptibility against eight antibiotics.

Isolate	Gentamycin	Ciproflox acin	Ampicillin	Oxacillin	Cefoitin	Cefotaxime	Ceftazidome	Ceftriaxone
C. Jej	NA	NA	NA	NA	NA	NA	NA	NA
E. coli	Sensitive	Sensitive	Intermediate	Resistant	Sensitive	Sensitive	Sensitive	Sensitive
Salmo	NA	NA	NA	NA	NA	NA	NA	NA

Susceptibility of *E.coli* against antimicrobial agents

- All the 40 isolates were susceptible to gentamycin, ciprofloxacin, cefoxithin & cefotaxime.
- 39 (97.5%) isolates were sensitive, 1(2.5%) was resistant to ceftazidime & ceftriaxone.
- Isolates were sensitive 23 (57.5%) intermediate 10 (25%) & resistant 7(17.5%) to ampicillin.
- All isolates were resistant to oxacillin.
- 6 (15%) isolates were resistant to both Ampicillin & Oxacillin.

Resistance patterns of *E.coli* in the different samples

- 37 (100%) isolates from fecal samples were susceptible to gentamycin, ciprofloxacin, cefoxithin &cefotaxime.
- All the 37 (100%) isolates were resistant to oxacillin.
- 22 (59.5%) isolates were sensitive, 9 (24.3%) with intermediate & 6 (16.2%) were resistant to ampicillin.
- 36 (97.3%) isolates were sensitive 1(2.7%) was resistant to both ceftazidime & ceftriaxone.
- All (3) isolates from water were sensitive to gentamycin, ciprofloxacin, cefoxithin, cefotaxime, ceftazidime & ceftriaxone.
- All 3 isolates from water were resistant to oxacillin.
- In case of ampicillin, 1(33.3%) isolate was sensitive, 1(33.3%) was resistant & 1(33.3%) was intermediate.

Susceptibility	Gentamycin	Ciprofloxacin	Ampicillin	Oxacillin	Cefoxitin	Cefotaxime	Ceftazidime	Ceftriaxone
Fecal								
Sensitive	37(100%)	37(100%)	22	0	37(100%)	37(100%)	36	36
Intermediate	0	0	9	0	0	0	0	0
Resistant	0	0	6	37(100%)	0	0	1	1
Water								
Sensitive	3 (100%)	3 (100%)	1	0	3 (100%)	3 (100%)	3 (100%)	3 (100%)
Intermediate	0	0	1	0	0	0	0	0
Resistant	0	0	1	3 (100%)	0	0	0	0

Discussion

- *E. coli* was isolated from the 184 samples collected from the 33 study farms.
- Bacteria including foodborne pathogens, opportunistic pathogens or commensals can develop AMR (Teuber, 2001).
- Although the current study did not conduct molecular characterization of *E. coli* to confirm that the isolates were pathogenic, the findings are important for efforts to improve public health by preventing AMR, commensal bacteria in food producing animals can be reservoirs of antibiotic resistance genes (Knezevic and Petrovic, 2008).
- Increases in AMR in *E. coli* have been paralled by an increasing incidence of *E.coli* associated sepsis suggesting a possible link between resistance and virulence (Johnson *et al*., 2004).

Discussion

- Humans can become infected by *E. coli* of animal origin and may cause infections in humans with limited therapeutic options leading into treatment failure besides transferring AMR genes to other pathogenic *E. coli* strains.
- In humans, *E. coli* may cause several infections including GIT infections associated with diarrhea, UTIs, meningitis, peritonitis, septicemia & gram negative bacterial pneumonia (Haemmerum & Heuer, 2009).
- All 40 isolates were susceptible to gentamycin, ciproflaxacin, cefoxitine and cefotaxime, are still effective for treating *E.coli* associated diarrhea & other enteric infections.
- 6 isolates were resistant to AMP & OXA, treatment may be associated with high treatment failures.
- All the isolates, whether isolated from fecal or water samples were resistant to Oxacillin, use of this agent is likely to result into treatment failure.

Conclusions

- *All E. coli* isolates from fecal and water samples showed high resistance towards oxacillin.
- Four antibiotics (gentamycin, ciproflaxacin, cefoxitine and cefotaxime) were effective against *E. coli*.
- Campylobacter and Salmonella were not detectable in the study area.

Limitations

- Lack of Funding for the molecular characterization of the study isolates and genotyping of the resistant and virulence genes.
- We shall address the limitation through sourcing for funding to carryout the molecular characterization.

Recommendations

- Suspend routine use of oxacillin in treating bacterial infections.
- Restrict access and use of gentamycin, ciproflaxacin, cefoxitine and cefotaxime by the farmers to prevent or delay emergence of resistance.
- Implement public education programs targeting consumers, clinicians & dispensers of antibiotics to promote good stewardship & rational use of antimicrobial agents

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