The Nelson Mandela AFrican Institution of Science and Technology

NM-AIST Repository | https://dspace.mm-aist.ac.tz

Life sciences and Bio-engineering | Research Articles [LISBE]

2016-09-30

# Bacterial contamination of pork carcasses from Arusha, Tanzania

Luanda, Catherine

Global Journal of Advanced Research

http://dspace.nm-aist.ac.tz/handle/123456789/68

*Provided with love from The Nelson Mandela African Institution of Science and Technology*

Vol-3, Issue-9 PP. 806-817 ISSN: 2394-5788

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

# BACTERIAL CONTAMINATION OF PORK CARCASSES FROM ARUSHA, TANZANIA

**Ms Catherine M. Luanda, Joram Buza, Gaspary Mwanyika, Beatus Lyimo & Rehema Mrutu**
Nelson Mandela African Institute of Science and Technology, 4477 Arusha,
Tanzania.
luandac@nm-aist.ac.tz, joram.buza@nm-aist.ac.tz, mwanyikag@nm-aist.ac.tz,
lymob@nm-aist.ac.tz, mrutur@nm-aist.ac.tz

**Douglas R. Call & Murugan Subbiah**
Paul G. Allen School for Global Animal Health, Washington State University,
Pullman, Washington, 99164-7090.509-335-2489;
USA.
drcall@vetmed.wsu.edu, msubbiah@vetmed.wsu.edu

## ABSTRAC T

Consumer demand for pork in Tanzania is driving growth of an increasing number of poorly equipped formal and informal slaugther facilities. To assess the potential public health challenges from these developments, we assessed the magnitude of microbial contamination of pork carcases from slaughterhouses in urban Arusha. Carcasses surface swabs (n = 90) from three slaughterhouses (designated A, B and C with carcass turnover of 1-30, 30-50 and 50-100 per day, respectively) were sampled and *E. coli* and *Salmonella* isolates (n = 1,632 and n = 177, respectively) were tested against a panel of 11 antibiotics using breakpoint assays. On average, *E. coli* and *Salmonella* were recovered from 71.1% and 66.7% of carcasses, respectively. Prevalence was highest (*E. coli*, 90.0% and *Salmonella*, 90.0%) for carcasses from site B, a facility where all slaughter operations, including eviscerations, were completed on the floor. The lowest prevalence (*E. coli*, 43.3% and *Salmonella*, 36.7%) was recorded for carcasses from Site C, a facility where most operations were completed with the carcasses hanging from overhead rails. Resistance to at least one antibiotic was observed in 71.2% of the *E. coli* isolates, most commonly to ampicillin (53.2%) followed by amoxicillin (38.8%) and sulfamethoxazole (31.0%). Approximately 45.2% of *Salmonella* isolates were resistant to at least one antibiotic while 23.2% were resistant to two or more of the tested antibiotics. Resistance to streptomycin (19.8%), trimethoprim (18.6%), ampicillin (16.9%) and sulfamethoxazole (13.6%) were most common. All isolates were susceptible to cefotaxime, ceftazidime, and chloramphenicol. These findings associate poor slaughter practices with bacterial carcass contamination and provide evidence for potential to contract antimicrobial resistant *E. coli* and *Salmonella*..

**General Terms:** Microbial load, multidrug resistant phenotypes, slaughterhouses, antimicrobial resistant bacteria

**Keywords:** Antibiotic resistance, pork carcasses, health hazards, *E. coli*,*Salmonella*

## 1. INTRODUCTION

Demand for food animal products is increasing world wide. Tanzania, a developing East African country, is striving to meet this growing demand while also improving the nation's economy through export markets [1]. Meat production in Tanzania increased from

378,500 to 449,673 tonnes between year 2005 and 2010 [2]While positive from an economic standpoint, the increase also represents opportunities for uncontrolled local market growth where the quality of meat may be compromised from microbiological contamination. Zoonotic transmission of disease agents can cause serious illness to people handling and consuming such contaminated products[3]. Improving the quality of livestock production and meat processing are key to reducing microbial contamination of meat products[4, 5].

Livestock production forms a large percentage of agricultural activities and is an integral part of the economy in most African countries.Swine represent a small (3.7%) but growing component of the total livestock resources in Tanzania, which includescattle, sheep, goats and poultry. The swine population in Tanzania increased from 1.13 million head in 2003 to approximately 2.1 million by the end of 2008[6], representing a net increase of 6.7%[7]. Consumption of food animal products between 2005 and 2009 increased by 6.3% in Tanzania with swine having the largest proportional increase [1].

Despite the increase in demand for pork products, the meat processing industry in Tanzania is largely underdeveloped[8] with continuing risks of compromised food safety[9,10,11]. Informal pig slaughter slabs have proliferated and these facilities were the target of a 2014 government crackdown on unregistered slaughterhouses in the country[12]. Increased consumer demand and commensurate increased handling volume at registered slaughterhouses puts these facilities at risk for lower hygiene practices[13,2].

Swine harbor several parasitic, bacterial and viral infectious agents, the majority of which are zoonotic [14]. In Tanzania these include *Brucella*, *Salmonella*, *Leptospira*, *Mycobacterium*, hepatitis E, ascaris and *Trichonella*[15]. Both consumers and animal handlers are at risk and poor slaughter practices lead to a higher probability of carcass contamination with other intestinal bacteria. For instance, a study of thermophilic*Campylobacter* in pigs slaughtered at Morogoro slaughter slabs revealed that 12.2% of the *Campylobacter*-positive carcasses were from *Campylobacter*-positive live pigs [11]. Furthermore, meat-borne pathogens may harbor antibiotic resistance traits thus their transmission to people may propagate the spread of antibiotic resistance. A recent survey of antimicrobial-resistant bacteria in private Tanzanian hospitals found that 45.5% of clinical specimens were positive for bacteria that were resistant to at least two antibiotics[16]. Vila *et al*., [17] reported that in the Ifakara region of Tanzania 38% of diarrheagenic *E. coli* from children <5 years were multi drug resistant.To assess the potential that pork products might contribute to the disease burden in Tanzania,we estimated the prevalence of *E. coli* and *Salmonella* from pork carcasses from Arusha, Tanzania and characterized the recovered isolates for antibiotic-resistance phenotypes.

## 2. MATERIALS AND METHODS

### 2.1 Sampling Sites and Sampling Procedures

To estimate the bacterial load [colony forming units per $cm^2$ ($CFU/cm^2$)], 30 carcasses were sampled at three registered swine slaughterhouses in Arusha. These sites are located approximately 7.4 km apart from each other and sampling occurred during normal business hours between June 10th and July 27th 2015. To maintain anonymity, the slaughterhouses were coded as sites A, B and C. On average 1-30 pigs were slaughtered per day at site A, 30-50 at site B, while site C had the capacity to slaughter 50-100 pigs per day. Although all three sites implemented a basic process flow (bleeding, scalding, dehairing, head dropping, evisceration, sponging and hanging), it was not clear that procedures were undertaken to limit cross contamination [18]. At site C there was a prewash stage after carcass bleeding and a clear unidirectional flow with carcasses hanging from overhead rails. Sites A and B completed the slaughtering process with carcasses on the floor and without a prewash step. For these latter sites carcasses were only hung for sponging and weighing before the product was transported away from the facility.

### 2.2 Sample collection

During each sampling event, five immediately available carcasses were swabbed at the post-evisceration point on the processing line. We sampled at this point because it was the last common processing point for carcasses that were either returned directly to the producer or prepared for packing and retail sale. A total of 90 carcass surface swab samples were collected (30 each) using Pearce & Bolton's (2005) protocol with minor modifications. Briefly, 100 $cm^2$ of individual carcass surface was swabbed first using 50 $cm^2$ pre-moistened gauze pad that was previously soaked in 25 mL of sterile water contained in a 50 mL conical tube. A second 50 $cm^2$ pre-moistened gauze piecethatwas initially soaked in 25 mL of sterile Tryptic Soy broth (TSB, Becton, Dickson and company, Sparks, MD) was used to swab the carcass for recovery of *Salmonella*. A fresh pair of nitrile gloves was used each time the swab sample was collected along the split midline from forequarter, shoulder, rack, tenderloin to leg with chump. Both halves of the split carcass were

swabbed. After swabbing pads were put back into the original conical tubes and were transported on ice to the laboratory at Nelson Mandela African Institution of Science and Technology (NM-AIST, Arusha, Tanzania).

## 2.3 *E. coli* culture

Gauze pads were washed well in the original aliquot of sterile water by vigorous shaking. An aliquot (1 mL) was mixed with glycerol (15% final concentration) and stored at $^{-}80^{\circ}C$ for future use. Another aliquot (100 µl) of the wash was added into 900 µl of sterile distilled water to make 10-fold dilutions. From each dilution (diluted to $10^{-3}$), 100 µl wash was plated independently onto MacConkey agar plates (MAC, Becton, Dickson and Company, Sparks, MD) and spread uniformly using sterile glass beads to obtain isolated colonies. The inoculated MAC plates were incubated overnight (16-18 h) at $37^{\circ}C$ before counting presumptive *E. coli* colonies. *E. coli* was identified as pink to dark-rose lactose fermenting colonies. Basic identification in this fashion has been sufficient for >95% correct identification based on PCR testing for the *uidA* gene [19]. If colony density was too high (>300/plate), the stored samples were further diluted and the process was repeated. The CFU from the original wash was calculated and transformed to yield $\log_{10}(CFU/cm^2)$.

## 2.4 Estimating *E. coli* load from carcass surfaces

To determine the prevalence of antibiotic-resistant *E. coli* on the surface of swine carcasses, up to 24 presumptive *E. coli* isolates per sample were picked from the MAC plates using sterile toothpicks and were inoculated individually into 150 µl of Luria-Bertani broth (LB broth, Becton, Dickson and Company, Sparks MD) contained in the wells of 96-well microtitre plates. These inoculated plates were wrapped in cling-wrap and incubated overnight at 37˚C. After incubation a duplicate 96-well culture plate was made from the original 96-well culture plate for antibiotic susceptibility testing. Glycerol (15% final concentration) was added to each well of the original plate and stored at $^{-}80^{\circ}C$. The biochemical identity of presumptive *E. coli* colonies was further confirmed by transferring each plate of isolates onto Hi-Chrome agar (HC, Becton, Dickson and Company, Sparks, MD) (150 mm, diameter plates) using the sterile 96-pin replicator. Plates were incubated overnight at $37^{\circ}C$ and bluish-green colonies were considered to be *E. coli*. Importantly, while simple colony morphology testing with MAC agar is sufficient for >95% accuracy for *E. coli* identification [19], the additional testing with Hi-Chrome agar provided a greater degree of confidence in our conclusions.

## 2.5 *Salmonella* culture and isolation

Gauze pads were washed in the remaining 25 mL sterile TSB broth in the sampling conical tube by vigorously shaking. An aliquot (1 mL) was mixed with glycerol (15% v/v, final concentration) and stored at $^{-}80^{\circ}C$ for future use. The broth was then incubated overnight (16-18 h) at $37^{\circ}C$ for non-selective enrichment. After 24 h approximately 10µl of the culture was inoculated onto Xylose Lysine Deoxycholate Agar (XLD, Hi Media Laboratories Pvt. Ltd, Mumbai, India) to obtain isolated colonies. The inoculated XLD plates were incubated overnight (16-18 h) at $37^{\circ}C$. Plates were then examined for well-isolated, presumptive *Salmonella* colonies (red colonies with black centers). If no well-isolated colonies were present, frozen enrichment culture was thawed and serially diluted and plated on agar to obtain well-isolated colonies. Three presumptive *Salmonella* colonies were picked from each plate for biochemical confirmation on Triple Sugar Iron Agar (TSI, Hi Media Laboratories Pvt Ltd, Mumbai, India). A slant agar tube was prepared by tilting and holding molten TSI agar in a test tube and allowing it to solidify forming a slant surface at the top and a column at the bottom. A sterile straight wire loop was used to pick the center of presumptive colonies for inoculation onto slant surface and then the bottom column of the TSI agar. The tubes were then incubated overnight (16-18 h) at 37˚C. After incubation the tubes appearing red at the slant surface (alkaline) and yellow bottom (acidic) with bubbles (gas) and black residue ($H_2S$) were considered *Salmonella.*

## 2.6 Determining the prevalence of antibiotic resistant *E. coli* and *Salmonella* isolates

Each biochemically confirmed *E. coli* and *Salmonella* isolate was tested for susceptibility against a panel of 11 antibiotics that included amoxicillin (Amx, 32 µg/ml, MP Biomedicals LLC, Solon, OH), ampicillin (Amp, 32 µg/ml, VWR International LLC, Sanborn, NY), cefotaxime (Ctx, 8 µg/ml, Chem-Impex International INC, Wood Dale, IL), ceftazidime (Ceft, 8 µg/ml, SIGMA-ALDRICH Co., St. Louis , MO), chloramphenicol (Chl, 32 µg/ml, Medaitech Inc., Manassas, VA), ciprofloxacin (Cip, 4 µg/ml, Enzo Life Sciences Inc., Farmingdale, NY), gentamycin (16 µg/ml, Gen, Mediatech Inc.), streptomycin (Str, 64 µg/ml, AmrescoInc, Solon, OH), sulfamethoxazole, (Sul, 512 µg/ml, MP Biomedicals, LLC), tetracycline (Tet, 16 µg/ml, MP Biomedicals LLC), and trimethoprim (Tri, 8 µg/ml, MP Biomedicals LLC). Antimicrobial susceptibility testing was performed using a breakpoint assay (Subbiah*et al* 2011) with guidance from the Clinical Laboratory Standard Institute (2012). Briefly, each 96-well plate containing *E. coli* or *Salmonella* cultures was transferred onto MAC plates containing one of the antibiotics listed above and incubated overnight at 37ºC. Positive control strains NM-1 (resistant to Amp, Amx, Cfz, Ctx, Cip and Chl) and NM-2 (resistant to Gen, Str, Sul, Tet, and Tri)

Vol-3, Issue-9 PP. 806-817 ISSN: 2394-5788

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

and negative control strain K-12 (susceptible to all listed antibiotics) were inoculated onto each MAC plate. After incubation the plates were examined for bacterial growth indicated by formation of visible colonies; isolates that grew on MAC with antibiotics were considered resistant and coded "1" and those that did not grow were considered susceptible and coded "0". Isolates NM1 and NM-2 were originally isolated from water samples in Tanzania and were only used for quality control testing for the present study [20].

## 3. RESULTS AND DISCUSSION

### 3.1 Prevalence of *E. coli* and *Salmonella* positive carcasses

In total, 63 (71.1%) and 59 (65.5%) of the swab samples were positive for *E. coli* and *Salmonella*, respectively. Examination of the rank order for prevalence showed that site B had the highest prevalence for both bacteria (90% each) followed by site A, which processed the fewest carcasses on a daily basis. Slaughterhouse C, which used a carcass hanging rail system, had the fewest recoverable *E. coli* and *Salmonella* (Table 1).

**Table 1: Prevalence (%) of the *E. coli* and *Salmonella* positive carcasses sampled from swine slaughterhouses in the vicinity of Arusha, Tanzania. Results are based on 30 carcass swabs from each of three slaughterhouses.**

| Location | *E. coli*positive (n = 1,632) | *Salmonella*positive (n = 177) |
|---|---|---|
| A | 76.7% | 70% |
| B | 90.0% | 90% |
| C | 43.3% | 36.7% |

### 3.2 Antimicrobial resistance among *E. coli* isolates

A total of 1,632 *E. coli* isolates were collected from the three slaughterhouses (n = 671, 648, and 313 for sites A, B, and C, respectively). Overall, 1,162 (71.2%) of the isolates were resistant to at least one of the 11 antibiotics tested. The prevalence of resistance to at least one antibiotic was greatest for site B (87.2%, 95%CI = 0.845 to 0.896) followed by sites C (76.9%, 95%CI = 0.721 to 0.814) and A (53.1%, 95%CI = 0.493 to 0.568). The rank order for the most prevalent AMR phenotypes (>25%) at site AwasAmp, Amx, and Chl, which was different from both sites B (Amp, Amx, Sul, and Str) and C (Amp, Amx, Sul, Tet) (Table 2).

**Table 2: Average (%) prevalence of antibiotic resistant *E. coli* collected from swine carcasses slaughtered in three slaughter facilities in Arusha district, Tanzania.**

| Antibiotics | Average (%) ± Standard Error (SE) | | |
|---|---|---|---|
| | Site A<br>N = 671 | Site B<br>N = 648 | Site C<br>N = 313 |
| Ampicillin | 28.0 ± 0.07 | 71.0 ± 0.06 | 67.0 ± 0.07 |
| Amoxicillin | 27.0 ± 0.05 | 49.0 ± 0.05 | 42.0 ± 0.07 |
| Ceftazidime | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.3 ± 0.003 |
| Cefotaxime | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.3 ± 0.003 |
| Chloramphenicol | 26.0 ± 0.05 | 15.0 ± 0.04 | 15.0 ± 0.04 |
| Ciprofloxacin | 5.0 ± 0.02 | 7.0 ± 0.02 | 9.0 ± 0.04 |
| Gentamicin | 9.0 ± 0.02 | 1.0 ± 0.00 | 1.0 ± 0.04 |
| Streptomycin | 5.0 ± 0.03 | 29.0 ± 0.04 | 13.0 ± 0.03 |
| Sulfamethoxazole | 19.0 ± 0.05 | 39.0 ± 0.05 | 36.0 ± 0.04 |
| Tetracycline | 16.0 ± 0.03 | 7.0 ± 0.02 | 25.6 ± 0.05 |
| Trimethoprim | 6.0 ± 0.02 | 14.0 ± 0.03 | 11.2 ± 0.03 |

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

## 3.3 Antimicrobial resistance among *Salmonella* isolates

Of the 177 presumptive *Salmonella* isolates collected from the three slaughterhouses (n = 63, 81 and 33 for sites A, B, and C, respectively), 45.2% were resistant to at least one of the 11 antibiotics tested. Site B produced the highest average prevalence of isolates that were resistant to at least one antibiotic (70.4%, 95% CI= 0.598-0.795) followed by site A (25.4%, 95% CI = 0.159-0.371) and C (21.2%, 95%CI= 0.1-0.372). The magnitude of resistance to single antibiotics did not exceed 20% for the isolates tested in this study (Table 3). All isolates were susceptible to cefotaxime, ceftazidime, chloramphenicol and gentamycin.

**Table 3: Average (%) prevalence of antibiotic resistant *Salmonella* collected from swine carcasses slaughtered in three slaughter facilities in Arusha district, Tanzania.**

| **Antibiotics** | **Average (%) ± Standard Error** | | |
|---|---|---|---|
| | Site A<br>N = 63 | Site B<br>N = 81 | Site C<br>N = 33 |
| Ampicillin | 5.0 ± 0.03 | 32.0 ± 0.06 | 3.0 ± 0.03 |
| Amoxicillin | 2.0 ± 0.0 | 19.0 ± 0.05 | 3.0 ± 0.03 |
| Ciprofloxacin | 0.00 ± 0.00 | 0.00 ± 0.00 | 3.0 ± 0.03 |
| Streptomycin | 10.0 ± 0.05 | 26.0± 0.06 | 15.0 ± 0.09 |
| Sulfamethoxazole | 6.0 ± 0.03 | 26.0 ± 0.05 | 0.00 ± 0.00 |
| Tetracyclin | 5.0 ± 0.03 | 7.0 ± 0.03 | 15.0 ± 0.09 |
| Trimethoprim | 3.0 ± 0.02 | 37.0 ± 0.06 | 0.00 ± 0.00 |

All isolates were susceptible to cefotaxime, ceftazidime, chloramphenicol, and gentamicin
.

## 3.4 Multidrug resistance among *E. coli* isolates

A total of 139 unique multidrug resistant (MDR; =2 antibiotics) phenotypes were found in *E. coli* isolated from the three swine slaughterhouses (A =62, B =88 and C=59; Table S1). The highest prevalence of MDR was seen among isolates from site B (MDR = 69.9%) where as isolates from site A had the lowest MDR prevalence (31.9%). The prevalence of MDR isolates from site C was intermediate (59.1%) and some isolates from this site were resistant to as many as eight antibiotics. The largest number of isolates (287 in total) showed a combined resistance to two antibiotics, the most common combination being resistance to ampicillin and amoxicillin (84.7%). Very few isolates (n=27) were resistant to>6 antibiotics (Table 4). While all sites were positive for MDR phenotypes, no single MDR phenotype appeared to dominate at any of the sites. For example, the maximum prevalence at all sites was for Amp-Amx, but this was limited to between 6.4% and 13.1%. That is, these sites were characterized by a wide diversity of MDR phenotypes rather than by a few highly prevalent phenotypes (Table S1).

## 3.5 Multidrug resistance among *Salmonella* isolates

The prevalence of MDR among *Salmonella* isolates (n=177) was 23.2%. A total of 25 unique MDR phenotypes were detected from the three sites sampled (A =3, B =20 and C=2) with the most frequent MDR resistance phenotype including streptomycin and tetracycline (2.82%; Table S2). The prevalence of MDR was highest for site B (40.7%) with three (8.02%) isolates being individually resistant to as many as five antibiotics. The prevalence of MDR was far less at site A (Table4). As with the *E. coli* isolates, at a population level the *Salmonella* isolates were characterized as having very diverse antibiotic-resistance phenotypes with little evidence that any single phenotype was dominant.

**Table 4:** **Mean prevalence (%) of multidrug resistant *E. coli* and *Salmonella* isolated from swine carcasses in Arusha district, Tanzania.**

| | Site (n= *E. coli* , *Salmonella*) | | |
|---|---|---|---|
| **Drug(s)** | A(n=671,63) | B(n=648,81) | C(n=313,33) |
| **0** | 46.8,74.4 | 16.5, 29.63 | 15.7,78.79 |
| **1** | 21.16,20.63 | 17.28, 29.63 | 17.57, 6.06 |
| **2** | 11.18, 4.76 | 19.44, 17.28 | 27.16, 12.12 |
| **3** | 6.41, 0.00 | 19.75, 9.88 | 9.58, 3.03 |
| **4** | 6.86, 0.00 | 18.52, 9.88 | 9.90, 0.00 |
| **5** | 4.02, 0.00 | 8.02, 3.70 | 4.15, 0.00 |
| **6** | 2.68, 0.00 | 3.55, 0.00 | 2.56, 0.00 |
| **7** | 0.45, 0.00 | 0.62, 0.00 | 4.15, 0.00 |
| **8** | 0.30, 0.00 | 0.00, 0.00 | 1.60, 0.00 |

n = number of isolates characterized from 30 swabs collected at each site

The prevalence of bacterial carcass contamination in the three swine slaughterhouses was high (71% for *E. coli* using direct plating and 66.7% for *Salmonella* using enrichment methods). This is approximately twice the prevalence that was reported by Bohaychuk*et al.,* [21] where 33.7% of pork carcass samples were positive for generic *E. coli* in Alberta, Canada. This degree of contamination by common enteric bacteria is noteworthy because the swabs were taken from pre-chilled carcasses that were ready for final processing and delivery to consumers. Wheatley *et al*.,[22] reported that the total viable count of bacteria should decrease with each processing step after evisceration, suggesting that the counts may be considerably higher on the processing line and thus represent a potentially important occupational risk to workers [23, 24].

Carcasses from site B had the highest prevalence of bacterial carcass contamination for both *E. coli* and *Salmonella* (90% each) while the lowest prevalence was evident from site C (43.3% *E. coli* and 36.7% *Salmonella).* Only management at site C considered critical control points to minimize chances for cross contamination. These practices included hanging carcasses during processing, using different knives between operations, and washing tools using hot water between applications. Sites A and B did not formally restrict access to the slaughter floor and most of the slaughter steps (including throat slitting, head dropping, skinning and evisceration) were carried out with the carcasses sitting on the floors. Thus, poor slaughter practices likely contribute to the higher prevalence of contamination at sites A and B.
Antibiotic susceptibility testing revealed that 71.2% of *E. coli* (n =1,632) and 45.2% of *Salmonella* (n = 177) were resistant to at least one antibiotic. The majority of *E. coli* isolates were resistant to ampicillin (53.2%), amoxicillin (38.8%) and sulfamethoxazole (31.0%) while most (90%) were susceptible to third-generation cephalosporins. These results are consistent with antibiotic resistance information that has been published for clinical isolates in Tanzania (*21, 2, 12*). The distribution of resistance was different for *Salmonella* isolates with the most prevalent resistance including streptomycin (19.8%),trimethoprim (18.6%), ampicillin(16.9%) and sulfamethoxazole (13.6%), which is also consistent with findings from other studies [25].

Overall, 52.3% of all *E. coli* isolates were resistant to ≥2 antibiotics with the majority of these isolates (84.7%) being resistant to a combination of two antibiotics. Multidrug resistance was observed in 23.2% of the *Salmonella* isolates although the prevalence of resistance to any single combination of antibiotics was limited (<3%). This is similar to a report from Iran where 3.4% of *Salmonella* isolates were resistant to streptomycin and tetracycline among a collection of 58 multidrug-resistant clinical isolates [26]. Findings from this study reveal the current microbial status of pork from abattoirs in Arusha and highlight the potential health hazard for the spread of *E. coli* and *Salmonella* including antibiotic resistant strains. Fortunately, no resistance was detected for two mainline antibiotics (ciprofloxacin and chloramphenicol) that are used to treat certain types of salmonellosis in Tanzania[27].

High levels of carcass contamination and the occurrence of a wide variety of multidrug resistance phenotypes among these *E. coli* isolates indicates that slaughterhouses are possible“hotspots” for the dissemination of resistant bacteria into the environment. On average one pig can weigh between 60-90kg at the time of slaughter, and assessment of value chain for pork has shown that meat

leaving the slaughterhouses is unbiasly distributed to a broad market including, individual consumers, retail outlets like supermarkets, as well as mining and tourist companies [2]. Consequently, this represents a significant opportunity for intervention to improve practices at swine slaughter facilities and limit a potentially increasing public health hazard in the community.

## 4. CONCLUSION

Generally, the existing slaughter practices in pig slaughterhouses in Arusha put pork at higher risk of contamination by enteric bacteria as demonstrated by findings from the studies for *Salmonella* and *E. coli.* The wide variety of antimicrobial resistance phenotypes observed from these isolates also alerts to the potential role of pork in the spread of AMR and thus the potential health risks posed.

## 5. SUPPLEMENTAL INFORMATION

**Table 5. The (%) distribution of resistance phenotype of *E. coli* isolated from swine slaughterhouses in Arusha, Tanzania.**

| Antibiotic resistance phenotype | Overall<br>n = 1632 | Site A<br>n = 671 | Site B<br>n = 648 | Site C<br>n = 313 |
|---|---|---|---|---|
| Susceptible | 28.79 | 46.8 | 14.4 | 17.9 |
| Ampicillin (Amp) | 53.25 | 25.0 | 76.69 | 65.18 |
| Amoxacillin (Amx) | 38.85 | 23.5 | 53.55 | 41.21 |
| Ceftazidime(Cfz) | 0.18 | 0.0 | 0.3 | 0.32 |
| Cefotaxime(Ctx) | 6.8 | 5.0 | 7.56 | 8.95 |
| Ciprofoxacin (Cip) | 0.12 | 0.0 | 0.15 | 0.32 |
| Chloramphenicol(Chl) | 19.12 | 25.0 | 15.12 | 14.69 |
| Gentamycin(Gen) | 5.88 | 8.6 | 1.23 | 9.58 |
| Streptomycin(Str) | 17.09 | 5.22 | 32.25 | 11.18 |
| Sulfomethoxazole(Sul) | 31.0 | 17.6 | 43.67 | 33.55 |
| Tetracyclin(Tet) | 14.83 | 16.4 | 8.95 | 23.64 |
| Trimethoprim(Tri) | 10.42 | 5.67 | 15.74 | 9.58 |
| AmpAmx | 7.78 | 3.43 | 9.72 | 13.1 |
| AmpAmxCfzChlGenSulTet | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpAmxCfzCipStrSulTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxChl | 2.02 | 0.6 | 4.01 | 0.96 |
| AmpAmxChlGen | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxChlGenStrSulTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxChlGenSul | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxChlGenSulTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxChlStr | 2.02 | 0.6 | 4.01 | 0.96 |
| AmpAmxChlStrSul | 0.74 | 0.15 | 1.54 | 0.32 |
| AmpAmxChlStrSulTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxChlStrSulTri | 0.8 | 0.0 | 1.54 | 0.96 |
| AmpAmxChlStrTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxChlSul | 0.31 | 0.6 | 0.0 | 0.32 |
| AmpAmxChlSulTet | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpAmxChlSulTri | 0.55 | 0.15 | 0.77 | 0.96 |

GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

| | | | | |
|---|---|---|---|---|
| AmpAmxChlTet | 0.18 | 0.30 | 0.15 | 0.0 |
| AmpAmxChlTri | 0.43 | 0.15 | 0.93 | 0.0 |
| AmpAmxCip | 0.12 | 0.0 | 0.31 | 0.0 |
| AmpAmxCipChlGen | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpAmxCipChlGenStrSulTet | 0.25 | 0.15 | 0.0 | 0.96 |
| AmpAmxCipChlGenSul | 0.12 | 0.3 | 0.0 | 0.0 |
| AmpAmxCipChlGenSulTet | 0.67 | 0.15 | 0.0 | 3.19 |
| AmpAmxCipChlGenSulTetTri | 0.12 | 0.0 | 0.0 | 0.64 |
| AmpAmxCipChlGenTet | 0.18 | 0.3 | 0.0 | 0.32 |
| AmpAmxCipChlStrTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipChlSul | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpAmxCipChlSulTet | 0.12 | 0.0 | 0.15 | 0.32 |
| AmpAmxCipChlSulTetTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipChlSulTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipChlTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipChlTetTri | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpAmxCipGen | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpAmxCipGenSulTet | 0.31 | 0.15 | 0.15 | 0.96 |
| AmpAmxCipGenSulTetTri | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpAmxCipGenTet | 0.12 | 0.15 | 0.0 | 0.32 |
| AmpAmxCipStr | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipStrSulTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipStrSulTetTri | 0.12 | 0.0 | 0.31 | 0.0 |
| AmpAmxCipStrTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipSul | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipSulTet | 0.12 | 0.0 | 0.31 | 0.0 |
| AmpAmxCipSulTetTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipSulTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxCipTet | 0.12 | 0.0 | 0.31 | 0.0 |
| AmpAmxCtx | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxGen | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxGenStrSul | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpAmxGenSul | 0.25 | 0.3 | 0.15 | 0.32 |
| AmpAmxGenTetTri | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpAmxStr | 0.86 | 0.0 | 2.01 | 0.32 |
| AmpAmxStrSul | 4.29 | 1.19 | 8.95 | 1.28 |
| AmpAmxStrSulTet | 0.49 | 0.3 | 0.46 | 0.96 |
| AmpAmxStrSulTetTri | 0.49 | 0.6 | 0.31 | 0.64 |
| AmpAmxStrSulTri | 1.9 | 1.19 | 3.4 | 0.32 |
| AmpAmxStrTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpAmxStrTri | 0.18 | 0.0 | 0.46 | 0.0 |

| | | | | |
|---|---|---|---|---|
| AmpAmxSul | 4.47 | 1.94 | 7.56 | 3.51 |
| AmpAmxSulTet | 1.1 | 1.19 | 0.31 | 2.56 |
| AmpAmxSulTri | 1.35 | 0.3 | 1.54 | 3.19 |
| AmpAmxTet | 0.61 | 0.89 | 0.15 | 0.96 |
| AmpAmxTri | 0.12 | 0.0 | 0.31 | 0.0 |
| AmpCfzSul | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpChl | 1.04 | 0.6 | 1.08 | 1.92 |
| AmpChlGenTet | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpChlStr | 0.31 | 0.0 | 0.77 | 0.0 |
| AmpChlStrSul | 0.25 | 0.3 | 0.15 | 0.32 |
| AmpChlStrSulTet | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpChlSul | 0.86 | 1.94 | 0.0 | 0.32 |
| AmpChlSulTet | 0.18 | 0.45 | 0.0 | 0.0 |
| AmpChlSulTetTri | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpChlSulTri | 0.43 | 0.75 | 0.31 | 0.0 |
| AmpChlTet | 0.12 | 0.15 | 0.15 | 0.0 |
| AmpChlTetTri | 0.12 | 0.3 | 0.0 | 0.0 |
| AmpCip | 0.25 | 0.0 | 0.62 | 0.0 |
| AmpCipChlGen | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpCipChlGenStrSulTet | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpCipChlGenStrSulTetTri | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpCipChlGenSulTet | 0.55 | 1.19 | 0.0 | 0.32 |
| AmpCipChlGenSulTetTri | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpCipChlGenTetTri | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpCipChlStrSulTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpCipChlTet | 0.12 | 0.3 | 0.0 | 0.0 |
| AmpCipGen | 0.06 | 0.15 | 0.0 | 0.0 |
| AmpCipGenStrTet | 0.12 | 0.15 | 0.0 | 0.32 |
| AmpCipGenSul | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpCipGenSulTet | 0.55 | 1.19 | 0.0 | 0.32 |
| AmpCipGenSulTetTri | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpCipGenTet | 0.12 | 0.15 | 0.15 | 0.0 |
| AmpCipStrSul | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpCipStrSulTet | 0.18 | 0.0 | 0.46 | 0.0 |
| AmpCipStrTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpCipSul | 0.25 | 0.0 | 0.62 | 0.0 |
| AmpCipSulTet | 0.18 | 0.0 | 0.46 | 0.0 |
| AmpCipSulTetTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpCipTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpGen | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpStr | 1.04 | 0.15 | 1.39 | 2.24 |

| | | | | |
|---|---|---|---|---|
| AmpStrSul | 0.49 | 0.0 | 1.08 | 0.32 |
| AmpStrSulCtx | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpStrSulTet | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpStrSulTetTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmpStrSulTri | 0.31 | 0.0 | 0.46 | 0.64 |
| AmpStrTet | 0.18 | 0.0 | 0.46 | 0.0 |
| AmpStrTetTri | 0.06 | 0.0 | 0.0 | 0.32 |
| AmpSul | 2.14 | 0.3 | 3.4 | 3.51 |
| AmpSulTet | 0.31 | 0.0 | 0.15 | 1.28 |
| AmpSulTri | 0.43 | 0.0 | 0.31 | 1.6 |
| AmpTet | 0.92 | 0.15 | 0.31 | 3.83 |
| AmpTri | 0.18 | 0.0 | 0.46 | 0.0 |
| AmxChl | 0.61 | 1.34 | 0.0 | 0.32 |
| AmxChlGenStrSulTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmxChlSul | 0.06 | 0.0 | 0.15 | 0.0 |
| AmxChlTet | 0.12 | 0.3 | 0.0 | 0.0 |
| AmxCip | 0.06 | 0.0 | 0.15 | 0.0 |
| AmxCipTet | 0.06 | 0.0 | 0.15 | 0.0 |
| AmxGenStrSulTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmxStr | 0.06 | 0.0 | 0.0 | 0.32 |
| AmxStrSulTet | 0.12 | 0.0 | 0.0 | 0.64 |
| AmxStrSulTri | 0.61 | 0.15 | 1.39 | 0.0 |
| AmxStrTri | 0.06 | 0.0 | 0.15 | 0.0 |
| AmxSul | 0.12 | 0.0 | 0.15 | 0.32 |
| AmxSulTet | 0.06 | 0.0 | 0.0 | 0.32 |
| AmxTet | 0.98 | 2.24 | 0.0 | 0.32 |
| ChlGen | 0.43 | 1.04 | 0.0 | 0.0 |
| ChlGenTet | 0.06 | 0.15 | 0.0 | 0.0 |
| ChlSul | 0.31 | 0.6 | 0.15 | 0.0 |
| ChlSulTri | 0.12 | 0.15 | 0.15 | 0.0 |
| ChlTet | 0.37 | 0.89 | 0.0 | 0.0 |
| CipGenTet | 0.06 | 0.0 | 0.0 | 0.32 |
| CipStr | 0.06 | 0.0 | 0.15 | 0.0 |
| CipTet | 0.18 | 0.0 | 0.46 | 0.0 |
| StrSul | 0.31 | 0.0 | 0.77 | 0.0 |
| StrSulTetTri | 0.12 | 0.15 | 0.0 | 0.32 |
| StrSulTri | 0.18 | 0.15 | 0.31 | 0.0 |
| StrTet | 0.06 | 0.0 | 0.15 | 0.0 |
| StrTri | 0.06 | 0.15 | 0.0 | 0.0 |
| SulTet | 0.18 | 0.0 | 0.0 | 0.96 |
| SulTri | 0.31 | 0.45 | 0.0 | 0.64 |

**Table 6.** **The prevalence (%) of multidrug resistant *Salmonella* phenotypes isolated from swine slaughter houses in Arusha, Tanzania. Resistance to cefotaxime, ceftazidime, chloramphenicol and, gentamicin was not detected.**

| Antibiotic Resistance Phenotype | Overall N=177 | SITE A n=63 | SITE B n=81 | SITE C n=33 |
|---|---|---|---|---|
| Susceptible | 28.8 | 74.6 | 29.6 | 78.8 |
| Ampicillin (Amp) | 4.00 | 3.17 | 6.17 | 3.03 |
| Amoxacillin (Amx) | 2.82 | 1.58 | 3.70 | 3.03 |
| Streptomycin (Str) | 4.00 | 4.76 | 4.93 | 0.00 |
| Sulfamoxizole (Sul) | 3.40 | 4.76 | 3.70 | 0.00 |
| Tetracycline (Tet) | 1.69 | 3.17 | 1.23 | 0.00 |
| Trimethoprim (Tri) | 5.08 | 3.17 | 8.64 | 0.00 |
| AmpAmx | 1.13 | 0.00 | 2.47 | 0.00 |
| AmpAmxStrSul | 1.13 | 0.00 | 2.47 | 0.00 |
| AmpAmxStrSulTri | 0.00 | 0.00 | 1.23 | 0.00 |
| AmpAmxSulTri | 1.13 | 0.00 | 2.47 | 0.00 |
| AmpStr | 1.13 | 1.59 | 1.23 | 0.00 |
| AmpStrSulTet | 0.56 | 0.00 | 1.23 | 0.00 |
| AmpStrSulTetTri | 1.13 | 0.00 | 2.47 | 0.00 |
| AmpStrSulTri | 1.69 | 0.00 | 3.70 | 0.00 |
| AmpStrTet | 0.56 | 0.00 | 1.23 | 0.00 |
| AmpStrTri | 0.56 | 0.00 | 1.23 | 0.00 |
| AmpSul | 0.56 | 0.00 | 1.23 | 0.00 |
| AmpSulTri | 0.56 | 0.00 | 1.23 | 0.00 |
| AmpTri | 1.69 | 0.00 | 3.70 | 0.00 |
| AmxStrSul | 0.56 | 0.00 | 1.23 | 0.00 |
| AmxStrTri | 0.56 | 0.00 | 1.23 | 0.00 |
| AmxSulTri | 0.56 | 0.00 | 1.23 | 0.00 |
| AmxTri | 0.56 | 0.00 | 1.23 | 0.00 |
| CipStrTet | 0.56 | 0.00 | 0.00 | 3.03 |
| StrSul | 0.56 | 1.59 | 0.00 | 0.00 |
| StrSulTri | 1.13 | 0.00 | 2.47 | 0.00 |
| StrTet | 2.82 | 1.59 | 0.00 | 12.12 |
| StrTri | 2.26 | 0.00 | 4.94 | 0.00 |
| TetTri | 1.13 | 0.00 | 2.47 | 0.00 |

## 6. ACKNOWLEDGEMENTS

We acknowledge the government of Tanzania through the Nelson Mandela African Institute of Science and Technology, Paul G.Allen School for Global Animal Health (Washington State University) and the National Science Foundation (DEB1216040) for supporting this study.

## 7. REFERENCES

[1] Ministry of Livestock and Fisheries Development,2010. Livestock sector development strategy. .
[2] Wilson, R.T. and Swai, E.S.2014.Pig production in Tanzania: A critical review.

[3] Hershberger, E., Opera, S.F.,Donabedian, S.M.,Peri, M.,Bozigar,P.,Bartlett, P., and Zervos,M.J.2004.Epidemiology of antimicrobial resistance in *Enterococci* of animal originJournal of Antimicrobial Chemotherapy(2005)55,127-130

[4] Food and Agriculture Organization. 2008. Meat processing Technology for small-to medium-scale producers.

[5] Barros, M de A.F., Nero, A.L., Monteiro, A.A., and Beloti.V.2007.Identification of main contamination points by hygiene indicator microorganisms in beef processing plants. Food science and Technology.27,856-862

[6] National Bureau of Statistics, 2003. National Sample Census 2002/2003, Volume III: Small Holder Agriculture.

[7] National Bureau of Statistics, 2008. Tanzania Agriculture Sample Census 2007/2008,Volume III: Small Holder Agriculture

[8] Tanzania Meat Board. 2014. Investment opportunities in the meat industry.

[9] Mkupasi, E.M., Ngowi, H.A., and Nonga, H.E. 2010. Prevalence of extra-intestinal porcine helminth infections and assessment of sanitary conditions of pig slaughter slabs in Dar es Salaam city, Tanzania. Tropical Animal Health and Production. 43, 417-423

[10] Komba, E.V.G., Mkupasi, E.M., and Mbyuzi, A.O. 2012.Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: implications for public health. Tanzania Journal of Health Research. 14, 1-12

[11] Mdegela, R.H., Laurence, K., Jacob, P., and Nonga, H.E.2011. Occurrences of thermophilic Campylobacter in pigs slaughtered at Morogoro slaughter slabs, Tanzania. Tropical animal health and production. 43,83 -90

[12] GUARDIAN,Tanzania Ltd. 2014.TFDA shuts down Arusha abattoirs for operating in unhygienic conditions.

[13] Ministry of Livestock and Fisheries Development, 2006.National Livestock Policy-2006.

[14] Uddin Khan, S., Atanasova, K.R., Krueger, W.S., Ramirez, A. and Gray, G.C. 2013. Epidemiology, geographical distribution and economic consequences of swine zoonoses: A narrative review. Emerging microbes & infections.2,92

[15] Wilson, R.T., and Swai. E. 2013. A review of pig pathology in Tanzania. Tropical animal health and production. 45, 1269-1275

[16] Mwambete, K.D., and Nyaulingo, B. 2014. Antibiotic resistance profiles of bacterial pathogens from private hospitals, in Dar Es Salaam, Tanzania. International Journal of Pharmacy and Pharmaceutical Sciences.6,426-428.

[17] Vila, J., Martha, V., Casals, C., Urassa, H., Mshinda, H., Schellemberg, D. and Gascon, J.1999. Antimicrobial resistance of diarrheagenic*Escherichia coli* isolated from children under the age of 5 years from Ifakara, Tanzania. Antimicrobial agents and chemotherapy. 43, 3022-3024.

[18] FSIS-USDA, 1999.Generic HACCP Model for pork slaughter.

[19] Zhao, Z., Liu, J., Zhao, Z., Orfe, L.,Subbiah, M. and Call, D.2015. Soil-Borne Reservoirs of Antibiotc-Resistant Bacteria Are Established Following Therapeutic Treatment of Dairy Calves Soil-Borne Reservoirs of Antibiotc-Resistant Bacteria Dairy Calves.

[20] Lyimo, B., Buza, J., Smith, W., Subbiah, M. and Call, D.2016. Surface Waters in Northern Tanzania Harbor Fecal Coliform and Antibiotic Resistant *Salmonella* Spp. Capable of Horizontal Gene Transfer. African Journal of Microbiology Research.10,348-356

[21] Bohaychuk, V.M., Gensler, G.E. and Barrios, P.R. 2011. Microbiological baseline study of beef and pork carcasses from provincially inspected abattoirs in Alberta, Canada. The Canadian Veterinary Journal. 52,1095-1100

[22] Wheatley, P., Giotis, E.S. and McKevitt, A.I. 2014.Effects of slaughtering operations on carcass contamination in and Irish pork production plant. Irish Veterinary Journal.67,1-6

[23] Arrudha, P.S.2004.Swine Carcass Microbiological Evaluation and Hazard Analysis and Critical Control Points (HACCP) in a slaughterhouse in Minas Gerais, Brazil. Venezuelan Society of Microbiology.24, 83-88

[24] Hurd, H.S., Brudvig, J., Dickson, J., Mirceta, J., Polovinski, M., Matthews, N. and Griffith, R.2008.Swine Health Impact on Carcass Contamination and Human. Foodborne Risk. Public health reports.123,343-351

[25] Katakweba, A.A.S., Mtambo, M.M.A., Olsen, J.E. and Muhairwa, A.P. 2012. Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistant bacteria within livestock in Tanzania. Livestock Research for Rural Development. 24,170

[26] Firoozeh, F., Shahcheraghi, F., Zahraei, S.T., Karimi, V. and Aslani. M.M. 2011.Antimicrobial resistance profile and presence of class I integrons among *Salmonella enteric*serovars isolated from human clinical specimens in Tehran, Iran. Iranian Journal of microbiology.3,112-116

[27] Ministry of Health and Social Welfare. Standard Treatment Guidelines (STG) and the National Essential Medicines List(NEMLT) for Mainland Tanzania.2007.