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African Journal of Microbiology Research

*Full Length Research Paper*

# Recovery and prevalence of antibiotic-resistant *Salmonella* from fresh goat meat in Arusha, Tanzania

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**Meat products are clearly associated with foodborne pathogens including, antibiotic-resistant strains. Population growth and growing consumer demand facilitate the transmission of foodborne pathogens, particularly in developing countries. To determine the prevalence of antibiotic-resistant *Salmonella* in goat meat, a study was done in Tanzania (June to July, 2015). Overall 120 goat meat samples were collected from five large and five small slaughter facilities (n = 60, respectively). Pre-enrichment for *Salmonella* isolation was done in Tryptic Soy Broth followed by selective enrichment in Modified Semisolid Rappaport-Vassiliadis agar. Isolation of *Salmonella* was done in xylose-Lysine-Deoxycholate agar followed by biochemical confirmation in triple sugar iron agar. The average prevalence of *Salmonella* was 60 and 63% in large and small facilities, respectively. Breakpoint assays indicated an overall low prevalence of resistance (2 to 4%; n = 219 isolates) to ampicillin, amoxicillin, streptomycin, sulphamethoxazole and trimethoprim with complete susceptibility to ciprofloxacin, ceftazidime and cefotaxime. No significant difference ($p > 0.05$) in the prevalence of resistance between large and small facilities was observed. High probability of *Salmonella* contamination of goat meat from Arusha area of Tanzania can pose risks to consumers. Antibiotic resistance appears minimal in this population. Improved hygienic slaughter and meat-handling practices are encouraged to reduce the burden of *Salmonella*-positive meat products.**

**Key words:** Antibiotic resistance, goat meat, *Salmonella*, Arusha, Tanzania.

## INTRODUCTION

Meat, including goat meat, is an important source of protein but also can serve as a potential source of foodborne pathogens (Economou and Gousia, 2015). Meat products are typically contaminated during the slaughter process (Nouichi and Taha, 2009) with pathogens such as *Salmonella* sp., *Campylobacter* sp.,

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*Listeria monocytogenes* and pathogenic strains of *Escherichia coli* (Duffy et al., 2009; Gousia et al., 2011).

Salmonellosis is a common foodborne illness and is caused by a very diverse group of *Salmonella enterica* strains including *S. enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis (de Freitas Neto et al., 2010). Salmonellosis is responsible for approximately 155,000 worldwide deaths annually (Majowicz et al., 2010). Sub-Saharan regions in Africa bear the greatest toll of global foodborne disease burden where 70% of this burden is attributed to non-typhoidal *S. enterica* (Havelaar et al., 2015). People may acquire *Salmonella* infection either by consuming contaminated food products and water, or by direct contact with infected animals (Pui et al., 2011). Antibiotic-resistant *S. enterica* serovar Typhimurium strain type ST313 is a leading cause of bacteremia among African adults and children. In sub-Saharan Africa ST313 infection is associated with a case fatality rate of 20 to 25% (Feasey et al., 2012).

Use of antibiotics in food-animal agriculture is thought to contribute to the emergence and amplification of antibiotic-resistant strains of *Salmonella* (Economou and Gousia, 2015). Although, several published reports illustrate how antibiotic-resistant (ABR) bacteria isolated from food animals overlaps with those causing human infection including foodborne illness (Li et al., 2013; Marshall and Levy, 2011), non-overlap trends of antibiotic resistance infections between humans and animals are also reported (Mather et al., 2012). In developing countries, food and animal products pose a significant risk of transmission for both pathogens and antibiotic-resistant bacteria (Grace, 2015) in part because of unhygienic practices during slaughter and product processing (Mensah et al., 2012). Population growth and rising demand of goat meat in Tanzania for local and export markets has increased total goat meat production from 323,000 tons in 2000/2001 to 449,673 tons in 2009/2010 and consumption projections are still increasing (UNIDO, 2012). This trend may proportionately increase the risk of zoonotic infections associated with food animals in Tanzania. In addition, limited veterinary services and poor husbandry practices might contribute to risks of zoonotic disease transmission (Mellau et al., 2010).

In Tanzania, large slaughter facilities operate mainly in urban centers where meat condition and hygiene practices are inspected by municipal inspectors and health officers, respectively. These operations receive slaughter stock from local traders who purchase the animals either directly from local livestock keepers or obtain the animals from primary and secondary livestock markets. Small slaughter facilities are located in rural areas where inspection of meat and hygiene are uncommon. Small slaughter operators obtain slaughter stock from individual livestock keepers and primary livestock markets (NDO, 2008; UNIDO, 2012). In the Arusha area of Tanzania, roasted goat meat is a popular food-animal product, made famous as *Nyama Choma*. To study the potential contribution of goat meat in the transmission of ABR bacteria to workers and consumers, we estimated the prevalence of ABR *Salmonella* in fresh goat meat obtained from selected large and small slaughter operations in Arusha district.

## MATERIALS AND METHODS

### Sample collection

Ten (10) goat slaughter facilities were sampled between June and July, 2015. Depending on the number of animals slaughtered per day, the facilities were grouped into five large (A, B, C, D and E; 10 to 150 goat capacity per day) and five small (F, G, H, I and; 1 to 3 goat capacity per day) operations. Meat samples (n = 120, 250 g each) were purchased from five large (n = 60 samples; 12 per facility) and five small slaughter facilities (n = 60 samples; 12 per facility) and were placed in separate sterile polyethylene bags. Two to three samples were collected weekly from each facility over a period of 4 to 6 visits and were transported to laboratory in an ice-cold box within 2 h of collection. In the laboratory subsamples were excised (25 g each) and were washed thoroughly with 25 ml of double-distilled water in sterile plastic bags by soaking and vigorously shaking. An aliquot (1 ml) was transferred into a 2 ml micro-centrifuge tube containing glycerol (15% vol/vol, final concentration) and preserved at -20°C for future use if required. The remaining portion of meat wash was collected in a 15 ml falcon tube for non-selective pre-enrichment of *Salmonella* in the same day.

### Isolation and identification of *Salmonella*

An aliquot (1 ml) of meat rinsate was homogenized with 9 ml of Tryptic Soy Broth (TSB, Becton, Dickson and Company, Sparks, MD) and incubated overnight at 37°C to pre-enrich the culture for *Salmonella* (El-Aziz, 2013; Abakpa et al., 2015). After incubation, 30 µl of pre-enriched inoculum was dropped onto Modified Semisolid Rappaport-Vassiliadis (MSRV, Becton, Dickson and Company, Sparks, MD) agar plates containing 2% novobiocin (Becton, Dickson and Company, Sparks, MD). The plates were left to dry for 1 h and were then incubated at 42°C for 18 to 24 h. After incubation, the presumptive motile *Salmonella* were identified by the formation of whitish halos around the drops. Sterile loops were used to pick motile *Salmonella* from the periphery of halos and streak onto Xylose-Lysine-Deoxycholate Agar (XLD, HiMedia Laboratories Pvt, Ltd. Mumbai, India) and Xylose-Lysine-Tergitol 4 (XLT4) agar containing XLT4 agar supplement (Becton, Dickson and Company, Sparks, MD). The XLD and XLT4 plates were incubated at 35°C for 48 h. After incubation, the plates were observed for typical pink to reddish colonies with black centers on XLD plates that were considered presumptive *Salmonella* sp. and yellow to red colonies with black centers on XLT4 plates were identified as considered *S. enterica* serovar Typhimurium. Three isolates per sample were picked with sterile toothpicks and sub-cultured into 96-well plates containing 150 µl of LB broth (LBDifo$^{TM}$, Becton, Dickson and Company, Sparks MD). The plates were incubated at 37°C for 18 to 24 h. After incubation, 40 µl of glycerol (15% vol/vol final concentration) was added. All plates were stored at -20°C (Akbar and Anal, 2014; Lyimo et al., 2016).

### Confirmation of *Salmonella* identity by biochemical test

Presumptive *Salmonella* were confirmed by triple sugar iron (TSI) agar (TSI, Becton, Dickson and Company, Sparks, MD) using

previously-described procedures (Addis et al., 2011). Pre-enriched bacterial culture (for each sample in TSB) was inoculated onto TSI agar slants using a sterile inoculating loop and incubated overnight at 37°C. After incubation, colonies that showed yellow and red color changes in the butt and slope, respectively, with blackening of the slope due to production of hydrogen sulfide were confirmed as typical *Salmonell*a.

### Antibiotic resistance testing in *Salmonella*

Prior to antibiotic testing, the frozen 96-well culture plates were thawed at room temperature for approximately 20 min and a duplicate backup plate was prepared using a sterile 96-pin replicator. Antibiotic breakpoint assays were performed using procedures previously described (Tadesse et al., 2012) and antibiotic concentrations were prepared using recommendations guided by Clinical and Laboratory Standard Institute (CLSI, 2007). The following medically important antibiotics were included in the assays; ampicillin, 32 μg/ml (Amp, VWR International LLC, Sanborn, NY); amoxicillin, 32 μg/ml (Amx, MP Biomedicals LLC); chloramphenicol, 32 μg/ml (Chl, Medaitech Inc); ciprofloxacin, 4 μg/ml (Cip, Enzo Life Sciences Inc); ceftazidime, 8 μg/ml (Cfz, SIGMA-ALDRICH, St. Louis, MO); cefotaxime, 4 μg/ml (Cfx, Chem-Impex International LLC); gentamicin, 16 μg/ml (Gen, Mediatech Inc.); streptomycin, 16 μg/ml (Str, Amresco Inc); sulfamethoxaxole, 512 μg/ml (Sul, MP Biomedicals, LLC); tetracycline, 16 μg/ml (Tet, MP Biomedicals LLC); and trimethoprim, 8 μg/ml (Tri, MP Biomedicals, LLC).

About 1-2 μl of bacterial inoculum (~$10^4$ CFU per spot) was picked using a sterile 96-pin replicator and transferred onto MacConkey agar (MAC, Becton, Dickson and Company, Sparks, MD) plates containing each antibiotic. The plates were left to dry at room temperature for 15 min and were then incubated overnight at 37°C. After incubation the presence of antibiotic-resistant *Salmonella* was evident when an isolate grew on agar plate containing an antibiotic. *E. coli* K12 was used as negative control (susceptible to all tested antibiotics) and *E. coli* NM-1 (resistant to ampicillin, ciprofloxacin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline and trimethoprim) and *E. coli* NM-2 (resistant to ampicillin, amoxicillin/clavulanic acid, ceftazidime, ciprofloxacin, kanamycin, streptomycin, sulfamethoxazole, tetracycline and trimethoprim) were used as positive control organisms (Lyimo et al., 2016). NM-1 and NM-2 were originally recovered from water sources in Northern Tanzania and their resistance phenotypes were characterized at Nelson Mandela African Institution of Science and Technology (Arusha, Tanzania) and at Washington State University (Pullman, WA, USA) using the antibiotic breakpoint assays previously described (Rugumisa et al., 2016).

### Statistical analysis

All data were summarized by using descriptive statistics and tables and the difference in prevalence of *Salmonella* recovered from goat meat samples was assessed between large and small slaughter operations by using a two-sample Student's t-test. A comparison of prevalence of antibiotic-resistant *Salmonella* within and between large and small facilities across tested antibiotics was complete by using a two-factor analysis of variance (ANOVA) F-statistic and a TukeyHSD *post hoc* test (R software version 3.2.2). All results at $p < 0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

*Salmonella* was recovered from >50% of all meat samples with approximately 4% having traits consistent with *S. enterica* serovar Typhimurium. A low prevalence of *Salmonella* Typhimurium was also reported by others in other food animal products such as 11% in beef (Shilangale et al., 2015), 10.8% in cattle and sheep meat and 4.35% in poultry meat (Mezali and Hamdi, 2012). There was no significant difference ($p = 0.65$) in the prevalence between large and small facilities (Table 1). A high prevalence of *Salmonella* contamination in goat meat is consistent with deficits in the procedures used to process goats for meat. Qualitatively, it was observed that goats were held for a longer time in the secondary markets before being transported to slaughter facilities, increasing the chance of animal-animal contamination attributed to excretion while crowding of animals at waiting pans before slaughter might have contributed to more transmission of bacteria. In some areas, slaughter facilities were located closer to live goat auctions, presumably increasing the risk of further transmission. Furthermore, inadequate sanitation and cross-contamination of carcasses through the use of the unclean knives and other utensils for different animal carcasses and possibly the carriage of *Salmonella* sp., by personnel working at these facilities might be another source of carcass contamination.

These observations are consistent to findings reported in Australia by Duffy et al. (2009) who found that the prevalence of *Salmonella* contamination on goat carcasses varied from 3 to 45% in the morning and 20 to 40% in the evening hours, as a result of that which was attributed to poor handling practices. In Ethiopia, a high carriage (80%) of *Salmonella* sp*.,* was reported in various organs of apparently healthy slaughtered goats (Woldemariam et al., 2005) suggesting that carrier animals were potential sources of *Salmonella* contamination in slaughter facilities. In low-income countries like Tanzania, it is common to encounter inadequate food hygiene standards in meat production systems (Roesel and Grace, 2014). For the current study, the prevalence of non-Typhimurium *Salmonella* contaminated goat meat (>50% of carcasses) was higher than other published reports from Pakistan (8%), Ethiopia (3.8%) and Burkina Faso (7%) (Eze and Ivuoma, 2012; Kagambèga et al., 2011; Tadesse and Gebremedhin, 2015). This discrepancy may be attributed to inconsistent slaughter practices but might also be attributed to differences in pre-enrichment and isolation procedures employed across these studies. The low prevalence of serovar Typhimurium isolates (4%) is also consistent with the majority of contamination arising from environmental exposure during slaughter (Maharjan et al., 2006), which is an outcome that can clearly be addressed with greater attention to good hygiene practices. The majority of recovered *Salmonella* isolates (>96%) were susceptible to all of the tested antibiotics (Table 2) with no clear differences ($p > 0.05$) between large and small slaughter facilities (Table 1). Between large facilities, there was no

**Table 1**. Prevalence (%) of *Salmonella* positive goat meat samples and the % of antibiotic-resistant (ABR) *Salmonella* from selected large and small slaughter facilities in Arusha district, Tanzania.

| Slaughter facility | Positive samples (%) | Number of *Salmonella* isolated | ABR (%) |
|---|---|---|---|
| **Large facility (n=5)** | | | |
| A | 50 | 18 | 11.1 |
| B | 58.3 | 21 | 28.6 |
| C | 50 | 18 | - |
| D | 75 | 27 | 29.6 |
| E | 66.7 | 21 | 4.8 |
| **Overall mean** | 60±4.9[a] (50.5-69.5)[b] | 21.2±1.6[a] | 18.5±6.2[a] |
| **Small facility (n=5)** | | | |
| F | 75 | 27 | 7.4 |
| G | 75 | 27 | 14.8 |
| H | 58.3 | 21 | 9.5 |
| I | 58.3 | 21 | 4.8 |
| J | 50 | 18 | 38.9 |
| **Overall mean** | 63.3±5.0[a] (53.5-73.1)[b] | 22.8±1.8[a] | 15.1±6.2[a] |
| ***p*-value** = 0.65 | | | |

[a] Values are means± standard errors; [b] 95% Confidence interval.

**Table 2.** Average prevalence (%) of antibiotic-resistant *Salmonella* in goat meat from five large and five small slaughterhouses in Arusha, Tanzania; a comparison by two way ANOVA F- statistic test and Tukey HSD *post hoc* test.

| Antibiotic | Large facility (n=5) | Small facility (n=5) | Overall mean |
|---|---|---|---|
| Ampicillin | 2.2±1.3 (-0.5-4.8) | 3.2±1.4 (0.5-5.9) | 2.9±0.5[A] |
| Amoxicillin | 0.95±0.95 (-0.9-2.8) | 0.95±0.95 (-0.9-2.8) | 0.82±0.03[A] |
| Streptomycin | 4.2±2.1(0.1-8.3) | 3.2±2.2 (-1.1-7.5) | 3.7±1.1[A] |
| Sulphamethoxazole | 1.95±1.3 (-0.5-4.4) | 3.6±2.0 (-0.4-7.6) | 2.9±0.5[A] |
| Trimethoprim | 1.2±1.2 (-1.2- 3.6) | 1.1±1.1 (-1.1-3.3) | 1.2±0.4[A] |
| **Overall mean** | 2.4±0.7[A] | 2.2±0.6[A] | |
| **F-statistic** | | | |
| Antibiotic | 0.02[ns] | | |
| Slaughter size | 0.38[ns] | | |
| Slaughter size*antibiotics | 0.04[ns] | | |

Values are means ± standard errors followed by different superscript letter (s) in the same column or raw which show significantly different groups by Tukey's honestly significant difference *post hoc* test at $P < 0.05$. [ns =] non-significant ($p > 0.05$). All tested isolates were 100% susceptible to ciprofloxacin, ceftazidime and cefotaxime. Chloramphenicol and tetracycline were not included due to insufficient data.

statistical difference ($p > 0.05$) in prevalence of antibiotic-resistant isolates (Table 3) while for small facilities there was a significant difference ($p < 0.05$) in prevalence that corresponded to a difference ($p = 0.044$) in the rank order of prevalence between sites J and I (Table 4). This difference may be associated with variation in the source of animals entering these facilities or a random difference.

The *Salmonella* isolates recovered for this study were mostly susceptible to antibiotics that are commonly used in Tanzanian livestock production [ampicillin, streptomycin and sulfamethoxazole (Katakweba et al., 2012)] suggesting limited selective pressures from the use of these antibiotics. The limited occurrence of antibiotic-resistant strains in *Salmonella* reported in this study is consistent for what has been reported for slaughtered goats in central Ethiopia where resistance to ampicillin, streptomycin and sulphamethoxazole varied

**Table 3.** Average prevalence (%) of antibiotic-resistant *Salmonella* in goat meat samples from different sites within large slaughterhouses in Arusha district, Tanzania; a comparison by two-way ANOVA F-statistic and Tukey HSD *post hoc* test

| Large facility (n=5) | Antibiotic[a] | | | | | Overall mean |
|---|---|---|---|---|---|---|
| | Amp | Amx | Str | Sul | Tri | |
| A | 0.53±0.53 | 0.53±0.53 | - | - | - | 0.21±1.5$^A$ |
| B | -[b] | - | 1.6±1.6 | - | - | 0.32±0.32$^A$ |
| C | - | - | - | - | - | - |
| D | 0.61±0.61 | - | 0.61±0.61 | 0.61±0.61 | 0.61±0.61 | 0.49±0.24$^A$ |
| E | - | - | 0.46±0.46 | 0.46±0.46 | - | 0.19±0.13$^A$ |
| **Overall mean** | 0.26 ±0.18$^A$ | 0.11 ± 0.11$^A$ | 0.49 ± 0.31$^A$ | 0.23 ±0.17$^A$ | 0.14 ±0.14$^A$ | |
| **F-statistic** | | | | | | |
| Antibiotic | 0.59$^{ns}$ | | | | | |
| Site | 0.86$^{ns}$ | | | | | |
| Antibiotic*Site | 0.70$^{ns}$ | | | | | |

Values are means ± standard errors followed by different superscript letter (s) in the same column or raw which show significantly different groups by Tukey honestly significant difference post *hoc* test at $p < 0.05$. [a] Amp, ampicillin; Amx, amoxicillin; Str, streptomycin; Sul, sulphamethoxazole and Tri, trimethoprim, All tested isolates were 100% susceptible to ciprofloxacin, ceftazidime and cefotaxime. -[b] = not detected; [ns] = non-significant ($p > 0.05$).

**Table 4.** Average prevalence (%) of antibiotic-resistant *Salmonella* in goat meat samples from different sites within small slaughterhouses in Arusha district, Tanzania; a comparison by two-way ANOVA F-statistic and Tukey HSD *post hoc* test.

| Small facility (n=5) | Antibiotic[a] | | | | | Overall mean |
|---|---|---|---|---|---|---|
| | Amp | Amx | Str | Sul | Tri | |
| F | 0.41±0.41 | - | - | 0.41±0.41 | - | 0.21±0.12$^{ABC}$ |
| G | 0.25±0.25 | - | - | 0.13±0.13 | - | 0.32±0.19$^{AC}$ |
| H | -[b] | 0.18±0.18 | - | - | - | 0.11±0.11$^{AB}$ |
| I | - | - | 0.16±0.16 | - | - | 0.09±0.09$^B$ |
| J | 0.21±0.21 | - | 0.42±0.42 | 0.42±0.42 | 0.21±0.21 | 0.93±0.42$^A$ |
| **Overall mean** | 0.41 ±0.24$^A$ | 0.12 0.12$^A$ | 0.41 ± 0.31$^A$ | 0.47 ±0.31$^A$ | 0.14 ±0.14$^A$ | |
| **F-statistic** | | | | | | |
| Antibiotic | 0.40$^{ns}$ | | | | | |
| Site | 2.5$^{*c}$ | | | | | |
| Antibiotic*Site | 0.49$^{ns}$ | | | | | |

Values are means ± standard errors followed by different letter(s) in the same column or row designate significantly different groups by Tukey's honestly significant difference post hoc test at $p < 0.05$. [a] Amp, ampicillin; Amx, amoxicillin; Str, streptomycin; Sul, sulphamethoxazole and Tri, trimethoprim. All tested isolates were 100% susceptible to ciprofloxacin, ceftazidime and cefotaxime. -[b] = not detected; [c] significant at $p < 0.05$; [ns] = non-significant ($p > 0.05$).

between 4.6 and 18.2% (Molla et al., 2006). In contrast, a high prevalence of antibiotic-resistant *Salmonella* to ampicillin (54.5%, amoxicillin (45.5%), streptomycin (81.8%), sulphonamide (42%) and Trimethoprim (75%) was reported for goat meat in eastern Ethiopia (Ferede et al., 2015). Presumable, these differences reflect differences in exposure to antibiotics, bacterial diversity and selection pressure in the natural environment (Sharma and Bist, 2010). Importantly, while a very low prevalence of antibiotic-resistant *Salmonella* in goat meat from Tanzania is observed, growing consumer demand in Tanzania (UNIDO, 2012) will result in increased production efforts and potentially an increasing reliance of antibiotics for food animal production and a potential commensurate increase in the prevalence of antibiotic-resistant *Salmonella*. Examples include reports of increased prevalence of *Salmonella* from poultry and beef that are resistant to fluoroquinolones and third-generation cephalosporins (Ahmed et al., 2014; Cabrera-Diaz et al., 2013; M'ikanatha et al., 2010). The increased use of antibiotics during animal breeding can introduce a selective pressure that leads to the development of resistance or even multi-resistance characteristics in some bacterial populations (Chen and Jiang, 2014).

Occurrence of *Salmonella* isolates that were resistant to ≥1 antibiotic was uncommon (10.5% of 219). Of these,

the most prevalent multidrug resistance at larger facilities included resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline (7.8%) and resistance to ampicillin, sulfamethoxazole and tetracycline (5.7%) in small facilities. This is dramatically different from the prevalence of multidrug resistant isolates (≥1 antibiotic) recently reported from water sources in northern Tanzania (88.5%) (Lyimo et al., 2016). If the lack of overlap in antibiotic resistance is an appropriate metric, then it is likely that goats are not a significant contributor to *Salmonella* contamination in these waters. In addition, the prevalence of multi-drug resistant *Salmonella* observed in this study is lower compared to prevalence reported from other food animals in Sudan (33.3 to 66.6%), Ethiopia (93.2%), United States (62%) and Korea (87.2%) (Fadlalla et al., 2012; Ferede et al., 2015; Khaitsa et al., 2007; Kim et al., 2012). This difference may suggest low antibiotics consumption in goat populations raised from Arusha area. Further studies are required to directly assess the impact of this practice. The present study indicates a high probability (>50%) that goat meat carcasses from the Arusha area are contaminated with *Salmonella.* Fortunately, antibiotic resistance strains were detected infrequently. Nevertheless, producers, processors and consumers should be alerted to better carcass and product handling practices to minimize the risk of *Salmonella* transmission from these products.

## Conflict of Interests

The authors declare that there is no conflict of interests.

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## Abbreviations

**ABR,** Antibiotic resistance; **CLSI,** Clinical and Laboratory Standard Institute; **NDO,** Netherlands Development Organization; **NMAIST,** Nelson Mandela African Institution of Science and Technology; **UNIDO,** United Nation Industrial Development Organization.

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