Alsalmi et al. | J Pure Appl Microbiol | 15(3):1634-1642 | September 2021 Article 6916 | https://doi.org/10.22207/JPAM.15.3.60

Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE



The Ecology and Antibiotic Resistance Patterns of Gastrointestinal Tract Infections in A Tertiary Care Hospital in Oman

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Abstract

A wide range of gastrointestinal (GI) illnesses is caused by foodborne bacteria that can arise from either a direct bacterial infection or bacterial toxin ingestion. The treatment of these infections has been hampered by the appearance of resistant strains. This current study aims to investigate the prevalence of Gastrointestinal tract (GIT) infections in Omani patients and their resistance pattern against commonly used antibiotics. Seven hundred and ninety fresh stool samples were obtained from Omani patients attending Sultan Qaboos University Hospital with GI manifestation from the 1st of June to the 30th of November 2019. Bacterial identification in stool samples was carried out by inoculation in culture media, microscopical examination and biochemical tests confirmed by MALDI. BD Phoenix[™]. The antibiotics sensitivity testing was carried out by the Manual disk diffusion method and by MALDI. BD Phoenix™. Out of 790 stool samples, 49 samples were positive for GIT bacterial infections. Salmonella spp. was the most prevalent isolate and more associated with children less than ten years old. Out of the 49 bacterial isolates, 3 (6.1%) were Clostridium difficili, 4 (8.2%) were Shigella flexneri, 5 (10.2%) were Campylobacter jejuni, and different Salmonella spp. serotypes were detected such as Salmonella Kentucky (8.2%), Salmonella enteritidis (6.1%), Salmonella infantis (4.1%), Salmonella welteverden (4.1%), Salmonella typhimurium (4.1%), Salmonella anatum (2.0%), Salmonella tesvia (2.0%), Salmonella Uganda (2.0%), Salmonella Arizona (2.0%) and (40.8%) of other Salmonella spp. serotypes. Eighty percent of isolated Campylobacter jejuni were resistant to Ciprofloxacin and Tetracycline. Salmonella spp. and Shigella flexneri were highly resistant to Amikacin, Gentamicin, and Cefuroxime. The low level of bacterial infection detected among screened patients in the present study indicates the excellent hand washing hygiene practice in reducing GIT infections among patients in Oman. This good hand washing hygiene

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(Received: March 04, 2021; accepted: August 13, 2021)

Citation: Alsalmi AAS, Al-Busafi SA, AL-Lamki RNA, Mabruk M. The Ecology and Antibiotic Resistance Patterns of Gastrointestinal Tract Infections in A Tertiary Care Hospital in Oman. J Pure Appl Microbiol. 2021;15(3):1634-1642. doi: 10.22207/JPAM.15.3.60

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practice is of great help in the efforts of controlling the spread of other severe diseases like COVID-19. However, detecting the emerging of antibiotic-resistant of GIT bacterial pathogens among patients in Oman, such as *Salmonella and Shigella* to a commonly used antibiotic such as Gentamicin, is alarming.

Keywords: Gastrointestinal tract infections, tertiary care hospital, Oman

INTRODUCTION

Diarrhoea resulting from GIT infections is a major health burden worldwide. Almost 4% of South Africa deaths during the year 2000 were deaths attributable to infectious diarrhoea, the fifth leading cause of death in this African country¹. Among children under the age of five, diarrhea ranks as the second leading cause of preventable illness².

Diarrhoea resulting from ingesting contaminated food or water. Person to person transmission can be the source of infection of various pathogenic bacteria, including *Escherichia coli, Shigella spp.*, non-typhoidal *Salmonella spp., Staphylococcus aureus, enterotoxigenic Bacteroides fragilis,* and *Campylobacter spp.* and *Enterococci faecalis*¹.

Enterococci faecalis have rapidly appeared as critical nosocomial pathogens, with increasing resistance to most, and some cases to all currently approved antibiotics³.

Both developed and less developed countries are affected by Gastrointestinal (GI) diseases, despite the strong relationship between GI illnesses and risk factors such as the absence of clean drinking water, poor sanitation, and other factors².

The emergence of resistant microorganisms is always a potential risk when antibiotics are administered⁴. One study to detect the extensive antibiotic resistance of diarrheagenic *Escherichia coli* and *Shigella spp.*, in local health centres of Isfahan province in Iran showed that 'bacterial dysentery due to *Shigella* species is considered to be the most complicated form of diarrhoea and a large number of associated deaths are reported annually⁵. The burden of diarrheal diseases is far more in developing countries for example in a multi-centre Asian study, the annual incidence of shigellosis was 13.2/1000 children under the aged of five years⁶. Furthermore, the antimicrobial resistance of enteric bacteria is rapidly increasing in the developing world. In the past, antibiotic resistance was mostly seen in nosocomial infections due to the widespread use of antibiotics in hospitals, however recently, multidrug-resistant bacteria are commonly encountered in community-acquired infections, particularly GIT infections. The globalization of antibiotics resistance is a reality⁷.

Moreover, an Iranian study which was performed to identify virulence genes and antibiotic resistance pattern of the enterotoxigenic and enteroaggregative *E. coli* isolated from stool samples obtained from diarrheagenic patients reveals that Gentamicin had the lowest resistance zero% while trimethoprim had the highest resistance (79.8 %)⁸. In this study, the resistance of *E. coli* isolates to *chloramphenicol*, cefotaxime, sulphamethoxazole, ciprofloxacin, ampicillin, and tetracycline were 3.5%, 7.01%, 71.05%, 10.5%, 52.63 %, and 3.5 %, respectively⁸.

A recent study was carried out in Qatar on 76 of E. coli obtained from less than ten years old diarrheagenic hospitalized children in order to identify the phenotypic resistance profiles and ESBL genotype and virulence profiles of Enteroaggregative E. coli (EAEC) and Enteropathogenic E. coli (EPEC)9. This study, and by using the E-test method showed that (73.7 %) of isolates were resistant to at least one antibiotic⁹. The isolates demonstrated overall, high resistance rate (70 %) to the firstline antibiotics such as ampicillin, tetracycline (46.4 %) and sulfamethoxazole-trimethoprim (42.9 %). Further, 39.5 % of the isolates from the stool samples obtained from ten years old diarrheagenic hospitalized children in Qatar, were multidrug-resistant (MDR), with 22.4 % being ESBL producers. On the other hand, all isolates were susceptible to fosfomycin, carbapenem, colistin and amikacin⁹.

Several studies were performed to detect antibiotic resistance of *Helicobacter pylori*. One of these studies was carried out in China from the year 2013 to 2016 and showed that the overall resistance rates were as follows: furazolidone (1.49%), amoxicillin (1.58%), rifampicin (2.80%), gentamicin (3.73%), doxycycline (9.20%), clarithromycin (22.73%), levofloxacin (24.75%), cefuroxime (25.20%), moxifloxacin (38.53%), ciprofloxacin (43.20%), ceftriaxone (49.60%), azithromycin (85.60%), tinidazole (87.20%), metronidazole (92.53%), ornidazole (94.27%) and cefetamet (97.20%)¹⁰.

A recent study was conducted to investigate the antimicrobial resistance rates and virulence gene profile among *Shigella* species isolated from stool samples obtained from Iranian children¹¹. The children aged between 2 months and 14 years. In this study, 141 were isolated as *Shigella spp.*, revealing *S. sonnei* as the most prevalent species (78.7 %), followed by *S. flexneri* (19.9 %) and *S. boydii* (1.4 %).

Antimicrobial susceptibility testing in this Iranian study, showed that most of the isolates (99.29%) were resistant to at least one antibiotic. A total of 101 (71.6%) *Shigella* isolates were multi drug resistant (MDR) strains¹¹.

The present study aims to assess the prevalence and characterization of GIT bacterial pathogens and detect its antibiotics resistance patterns among Omani patients attending Sultan Qaboos University Hospital (SQUH).

MATERIAL AND METHODS Specimens

This study was performed at SQUH, clinical microbiology laboratory. A total of seven hundred and ninety fresh stool samples was collected from patients having GI manifestation during the period between 1st of June and 30th of November 2019. Clinical data of each patient including sex, age, clinical history was collected. This project has been approved by the Medical Research Ethics Committee, College of Medicine & Health Sciences, Sultan Qaboos University, Muscat, Oman (SQU-EC/184/19).

Detection of pathogenic bacteria in stool samples using routine culture media

Briefly, 0.2 gram of fresh stool sample from each patient was mixed with one drop of saline and then smeared in a glass microscopic slide and heat-fixed on the hot plate. Once dried, the stool sample was examined under a light microscope to investigate for the presence of white blood cells, red blood cells. All findings were documented in the request form.

All stool samples were routinely cultured in Xylose Lysine Deoxycholate Agar (XLD), Hektoen Enteric agar (HE), *Campylobacter* agar (CAMPY), and selenite F broth. Moreover, stool sample inoculated in selenite F broth, were sub-cultured after 24 hours incubation at 37°C in XLD, HE and CAMPY agars. For detection of causative bacterial pathogens. XLD and HE agar plates were incubated for 24 hours at 37°C in aerobic conditions. CAMPY agar plates were incubated for 48 hours at 42°C in anaerobic conditions. Bloody stool samples were also cultured on a Sorbitol agar plate. After 24 hours of incubation of XLD and HE agar plate colonies with the yellow colour, which means that they ferment lactose, were considered as normal flora and reported as No Enteric Pathogen (NEP). Whereas, if any colorless colonies appear, subsequent identification tests were performed. Subsequent tests, including oxidase test, triple sugar iron (TSI) and urease. If oxidase negative results were obtained, urease and triple sugar iron (TSI) tests were carried out. Negative urease and TSI that show pink slant and yellow bottom with or without H2S formation (black color) suspect the presence of Salmonella or Shigella. The confirmation of bacterial identification was carried out by using MALDI system. Suspected colonies are sub-cultured in Cystine Lactose Electrolyte Deficient (CLED) agar plate (purity plate) for subsequent identification and antibiotic sensitivity test using automated BD Phoenix[™]. Once Salmonella spp. is confirmed, a sample was sent to Central Public Health Laboratories (CPHL), Muscat, for serotyping. After 24 hours of incubation of stool samples, Campy agar plate is retrieved, Campylobacter spp. growing colonies appear as a grey in coloured colonies with a shape like a water droplet and have aluminium

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shin. Gram staining of *Campylobacter spp.* reveals curved gram-negative bacteria, which is oxidase positive. Identification is *Campylobacter spp.* was confirmed by MALDI system.

Assessment of antibiotics sensitivity of bacterial isolate

Antibiotics susceptibility test was carried out on an automated BD Phoenix system, which provides rapid, accurate, and reliable detection of known and emerging antimicrobial resistance. Antibiotics sensitivity of bacterial isolate results were reported according to the Clinical and Laboratory Standards Institute (CLSI) 2019 recommendations. Manual disk diffusion method was also used in the present study for antibiotics susceptibility testing for *Campylobacter*, including Erythromycin, Tetracycline, Ciprofloxacin in the blood agar plate.

Data analysis

Patient's data were all documented in the Microsoft Excel program. Statistical analysis, resistance percentage, tables, and graphs were all done using the Microsoft Excel program 2010.

RESULTS

Clinical features

A total of 790 patients having GI manifestation was recruited in this study. Patients' age ranged from less than 1year to 92 years old

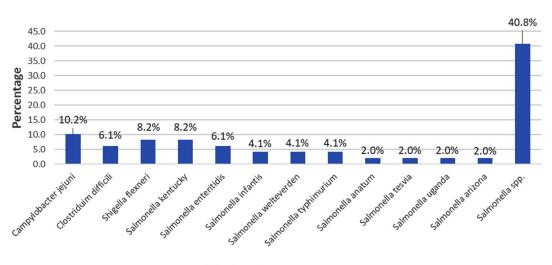
(Table 1). Of 790 patients, 414 were females & 376 were males (Table 2).

Pathogen occurrence

49(6.2%) stool samples out of 790 samples were positive for GIT infections. Out of 49 positive cases, 29(59.2%) were females, and 20 (40.8 %) were males. Out of the 49 bacterial isolates, 3 (6.1%) were *Clostridium difficili*, 4 (8.2 %) were *Shigella flexneri*, 5(10.2 %) were *Campylobacter jejuni*, and different *Salmonella spp*. serotypes were detected such as *Salmonella Kentucky* (8.2 %), *Salmonella enteritidis* (6.1 %), *Salmonella infantis* (4.1 %), *Salmonella welteverden* (4.1 %), *Salmonella typhimurium* (4.1%), *Salmonella anatum* (2.0 %), *Salmonella tesvia*

Table 1. Distribution of patients according to age

Age groups (years)	Number of cases	Percentage	
(0-10)	291	36.80%	
(11-20)	60	7.60%	
(21-30)	79	10%	
(31-40)	111	14.10%	
(41-50)	62	7.80%	
(51-60)	60	7.60%	
(61-70)	64	8.10%	
(71-80)	46	5.80%	
(81-90)	15	1.90%	
(91-100)	2	0.30%	



Enteric pathgens

Fig. 1. Distribution of enteric pathogens detected inpatient attending SQUH.

(2.0 %), Salmonella Uganda (2.0 %), Salmonella Arizona (2.0 %) and (40.8 %) of other Salmonella spp. serotypes. Fig. 1 shows the distribution of enteric bacterial pathogens detected in-patient attending SQUH with GI manifestations.

Association of GIT infection with gender

Out of the 29 bacterial isolates obtained from female stool samples, one sample (3.4 %) was positive for *Campylobacter jejuni*, 1 (3.4 %) was positive for *Clostridium difficili*, 4 (13.8 %) were positive for *Shigella flexneri*, and 23 (79.3 %) were positive for different serotypes of *Salmonella spp.* Out of the 20 bacterial isolates obtained from male stool samples, 2 (10 %) of them were positive for *Clostridium difficili*, 4 (20 %) were positive for *Campylobacter jejuni* and 14 (70 %) were positive for different serotypes of *Salmonella spp.* Fig. 2 and 3 show the distribution of enteric bacterial

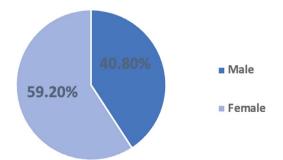


Fig. 2. Distribution of enteric bacterial pathogens in association with gender.

pathogens in both females & males patients diagnosed with GI manifestations.

Association of GIT infection with age

The majority of GIT infections (24 patients) detected were from less than ten years old age group and more associated with *Salmonella spp.* infections, followed by patients of the age group of (41-50) years old. Table 3 shows the distribution of enteric bacterial pathogens in association with different age groups.

Antibiotics resistance patterns

Antibiotics sensitivity testing of *Campylobacter jejuni*, shows that 80% of *Campylobacter jejuni* were resistant to Ciprofloxacin and Tetracycline, and completely sensitive to Erythromycin. *Salmonella spp.* show a high percentage of resistance to Amikacin, Gentamicin, and Cefuroxime (91.9 %, 89.2 %, and 86.5 %, respectively). *Shigella flexneri* show increased resistance to Amikacin (75 %), Gentamicin (75 %), Cefuroxime (75 %), and Ampicillin (75 %). Table 4 shows the prevalence of antibiotic resistance of *Campylobacter jejuni*. Table 5 describes the

 Table 2. Distribution of patients according to gender

Gender	Number of cases	Percentage	
Females	414	52.40 %	
Males	376	47.60 %	

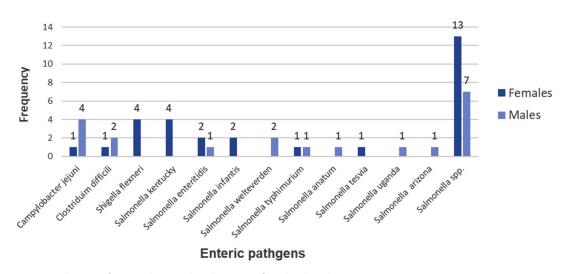


Fig. 3. Distribution of enteric bacterial pathogens in females & males.

prevalence of antibiotic resistance of *Shigella flexneri* and *Salmonella spp*. The prevalence of antibiotic resistance of *Salmonella spp*. serotypes are summarized in Table 6.

DISCUSSION

In this study, among 790 stool samples of patients attending SQUH with GI manifestations, 49 (6.2 %) were positive for bacterial GIT infections. This finding was fewer in terms of prevalence when compared with another study conducted in Thailand and showed that 84.9 % of GIT infection cases are associated with bacterial enteric infection¹².

In the present study, *Salmonella spp.* were the most prevalent bacterial isolate with a total of 37 (75.4 %), followed by *Campylobacter jejuni* 5 (10.2 %), *Shigella flexneri* 4 (8.2 %), *Clostridium difficili* 3 (6.1 %). These findings were consistent with a previous study performed at the University Hospital of Geneva in Switzerland¹³ to investigate the etiological agents of infectious diarrhoea in 7124 patient's specimens. This Geneva hospital study detected 369 (2.6 %) *Salmonella spp.*, 408 (2.9 %) *Campylobacter spp.*, and 79 (0.6 %) *Shigella spp.*¹³ In the present study, the association between GIT infections and sex of patients was investigated. GIT infections were prevalent in females more than males. Out of 49 positive samples, 29 (59.2 %) were females, and 20 (40.8 %) were males. A previous study carried out in Qatar showed a higher percentage of positive GIT infections among males (55.9 %) than females (44.2 %). This finding is in agreement with other findings in the Gulf region¹⁴.

The correlation between GIT infections and age was also investigated in the present study, the majority of positive cases 24 (49 %) were of age 10 years and below and 22 (91.6 %) of them were associated with *Salmonella spp.* infection. A previous study showed that the risk of *Salmonella* increases with age and patients infected with *Salmonella spp.* tended to be relatively older with a range of 85.8 years¹⁴.

The present study showed a high resistance percentage (80 %) of *Campylobacter jejuni* to Ciprofloxacin and Tetracycline and (0 %) resistance to Erythromycin. These results were consistent with a similar study from Belgium which showed high rates of resistance against Ciprofloxacin (55.8 %) and Tetracycline

Age groups (years)	Campylobacter jejuni	Clostridium difficili	Shigella flexneri	Salmonella spp	Total
(0-10)	2	0	1	22	24
(11-20)	1	0	0	2	3
(21-30)	2	0	1	0	3
(31-40)	0	1	1	3	5
(41-50)	0	1	0	8	9
(51-60)	0	1	0	1	2
(61-70)	0	0	1	1	2
(71-80)	0	0	0	0	0
(81-90)	0	0	0	0	0
(91-100)	0	0	0	0	0

Table 3. Distribution of enteric bacteria in association with age groups

Table 4. The prevalence of Antibiotic resistance of Campylobacter jejuni

Enteric pathogen / Antibiotics	Ciprofloxacin	Erythromycin	Tetracycline	
Campylobacter jejuni	80 %	0 %	80 %	

Enteric pathogen/ / Antibiotics														
	AK CN	ETM	MqI	MEM	CXM	CAZ	CRO) FEP	AMP	P AMC	IC TZP	P SXT		CIP
Shigella flexneri 7 Salmonella spp 91	75% 75% 91.9% 89.2%	% 0% % 0%	%0 80	%0	75% 86.5%	25% 5 13.5%	0% 6 16.2%	0% % 13.5%	75% 6 35.1%	6 0% % 2.7%	%0 %	6 25% 6 10.8%		25% 13.5%
AK: Amikacin, CN: Gentamicin, ETM: Ertapenem, IPM: Imipenem, MEM: Meropenem, CXM: Cefuroxime, CAZ: Ceftazidime CRO: Ceftriaxone FEP: Cefepime AMP: Ampicillin, AMC: Amoxicillin-Clavulanate, TZP: Piperacillin-Tazobactam, SXT: Trimethoprim-Sulfamethoxazole, CIP: Ciprofloxacin.	micin, ETM: ate, TZP: Pipe	Ertapenem eracillin-Tazo	, IPM: Imil bactam, SX	penem, MI (T: Trimetho	EM: Merol pprim-Sulfa	penem, CX imethoxazc	(M: Cefur ole, CIP: Ci	oxime, CAZ profloxacin	2: Ceftazid	ime CRO:	Ceftriaxo	ne FEP: Cé	efepime /	.MP: Ampicillin
Table 6. The prevalence of antibiotic resistance of <i>Salmonella spp.</i> Serotyp	e of antibiot	ic resistanc	ce of Salm	onella spp	n. Serotyp									
Enteric pathogen / Antibiotics	AK	CN	ETM	MqI	MEM	CXM	CAZ	CRO	ЕР	AMP	AMC	ТZР	SXT	CIP
Salmonella kentucky	100%	100%	%0	%0	%0	100%	25%	25%	25%	100%	%0	%0	25%	75%
Salmonella enteritidis	100%	100%	%0	%0	%0	100%	%0	%0	%0	%0	%0	%0	%0	%0
Salmonella infantis	50%	50%	%0	%0	%0	100%	%0	%0	%0	%0	%0	%0	%0	%0
Salmonella welteverden		100%	%0	%0	%0	100%	%0	%0	%0	%0	%0	%0	%0	%0
Salmonella typhimurium		100%	%0	%0	%0	100%	%0	%0	%0	%0	%0	%0	%0	%0
Salmonella anatum	100%	100%	%0	%0	%0	100%	%0	%0	%0	%0	%0	%0	%0	%0
Salmonella tesvia	100%	100%	%0	%0	%0	100%	%0	%0	%0	100%	%0	%0	%0	%0
Salmonella uganda	100%	100%	%0	%0	%0	100%	%0	%0	%0	%0	%0	%0	%0	%0
Salmonella arizona	100%	100%	%0	%0	%0	100%	%0	%0	%0	%0	%0	%0	%0	%0
Salmonella spp.	95%	%06	%0	%0	%0	80%	20%	25%	20%	35%	5%	%0	15%	10%

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(49.7 %). Alternatively, lower resistance rates to Erythromycin $(2 \%)^{15}$.

The present study detected high resistance percentage (75 %) of *Shigella flexneri* against Amikacin, Gentamicin, Cefuroxime, and Ampicillin, which is incomparable with a previously published Iranian study which showed that most frequent antibiotic resistance *Shigella flexneri* was towards Co-trimoxazole (89 %), Ampicillin (77 %) and Ceftriaxone (51 %), except for Ampicillin¹⁶. In the present study, *Salmonella spp.* similarly to *Shigella flexneri* show a high resistance percentage to Amikacin, Gentamicin, and Cefuroxime. This high resistance rate could be due to increased prescription of such antibiotics.

CONCLUSION

The present study showed that salmonella spp. is the most prevalent enteric bacteria in patients attending SQUH with a percentage of (75.4 %) and more associated with children of the age of 10 years and below. The second most prevalent was Campylobacter jejuni (10.2 %) followed by Shigella flexneri (8.2%), then Clostridium difficili (6.1 %). Both salmonella spp. and Shigella flexneri showed high resistance toward Amikacin, Gentamicin, and Cefuroxime. Campylobacter jejuni was highly resistant to Ciprofloxacin and Tetracycline.

This study demonstrated the antibiotic resistance patterns of bacterial pathogens isolated from fresh stool samples obtained from patients in Oman with GIT manifestations. It indicates the emerging of antibiotic-resistant of GIT bacterial pathogens such as *Salmonella* and *Shigella* to a commonly used antibiotic such as Gentamicin. This finding will be of great help to the health authorities in their monitoring of the antibioticresistant bacterial pathogens among cohort of patients in Oman.

ACKNOWLEDGMENTS

We are grateful to the head, the clinicians and the staff of the Microbiology Department, Sultan Qaboos University Hospital, Muscat, Oman for all of their help and support. The authors are grateful to the Central Public Health Laboratories, Muscat for the serotyping of the *Salmonella* spp. The authors are grateful to the Sultan Qaboos University ethical committee for allowing us to obtain patient data.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

AASA and RNA carried out the laboratory work. SAAB carried out the clinical supervision. MM conceived the presented idea and conducted the daily supervision of the project and wrote the manuscript. All authors reviewed and apporved the manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This project has been approved by the Medical Research Ethics Committee, College of Medicine & Health Sciences, Sultan Qaboos University, Muscat, Oman(SQU-EC/184/19).

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