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An assessment of Hygiene Indicator Bacteria and MDR *Salmonella* on Poultry Butchers' hands and Rinsing Water at XDR *Salmonella* Struck Areas

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FOOD-borne diseases are a major health problem in developing countries including Pakistan. With regard to meat hygiene, the question is posed whether carriers of pathogenic organisms, like *Salmonella, shigella,* and *E. coli* can contaminate a product with their hands. This might be possible in case of not using appropriate water, not maintaining proper hand hygiene during mean handling or due to bad toilet hygiene. This study was thus conducted to determine the prevalence of some selected hygiene indicator bacteria on the hands of poultry butchers and their hand washing water in two selected districts of Pakistan, i.e. Hyderabad and Jamshoro. Three hygiene indicator bacteria, *Salmonella*, *Shigella,* and *E. coli* were focused in this regard and for *Salmonella* spp. antibiotic resistance was also determine. Total 76 samples were collected (38 water and 38 hands). Isolation of bacteria were carried out by some standard microbiological techniques. Out of 38 water samples, 76.3%, 81.5% and 81.5% of samples were positive for *Salmonella, Shigella,* and *E. coli.* While for butchers' hand samples, the prevalence of *Salmonella, Shigella,* and *E. coli* was found as 92.1%, 97.3%, and 100%. The overall antibiotic resistance is as follows: ampicillin (89%), azithromycin (3.1%), ceftazidime (0%), gentamicin (25.5%), cefotaxime (25%), erythromycin (40.6%), neomycin (31.2%), streptomycin (48.4%), and sulphamethoxazole (50%). Percentage of Multi-drug resistant (MDR) isolates found in water and butchers' hands sample is 50% and 71.4%. One Extremely-drug resistant (XDR) isolate is also found in hands samples. The high prevalence of *Salmonella, Shigella, and E. coli* in butchers' hands and hand washing water can transfer to the meat and cause many foodborne infections in meat consumers.

Keywords: Butchers, Poultry, Hands, *Salmonella,* and Antibiotic resistance.

Introduction

In most cases, people pay more attention to the microbial quality of drinking water than the quality of water they used to wash their hands [1]. Hands play an important role in the spread and transmission of many pathogens that cause food borne diseases and nosocomial infections. The occurrence of foodborne infections is a major public health problem as it globally effects both developed and developing countries [2]. In developing countries, annually 2.2 million children die due to diarrheal diseases [3]. According to many authors food handlers carry the foodborne pathogens are asymptomatic [4, 5].

Hand washing is the act of cleansing the hands with water or any other liquid, with or without the use of soap or detergents, to ensure proper hygiene [6]. It is an aesthetic and hygienic practice that eliminates dead skin cells, organic material, sebum, sweat, temporary microorganisms and associated resident bacteria, that has adhered to your hands [7]. The importance of hand hygiene in the fight against infections should not be underestimated [2]. It is believed as one of the

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most effective ways to prevent foodborne illnesses as most of them are caused by microorganisms transmitted through contaminated hands [8]. Just as hand washing is important to reduce disease transmission, quality of water for hand washing is just as important for effective hand washing. The quality of water used for hand washing before, after or during food handling should be same as the water consumed by humans. It should not contain any fecal coliforms and pathogenic microbes [9]. The temperature of the water should also be considered and it has been suggested by Canadian Center for Occupational Health (CCOP) that it should be between 110 and 120°F (43 and 49°C) [7]. Hands that are not washed or rinsed with contaminated water pose a risk of contamination at higher level [10].

When it comes to the transfer of food-borne pathogens from butchers' hands to the human body, *Salmonella, Shigella,* and *E. coli* are of great importance. *E. coli* is a group of bacteria that live commensally in the gastrointestinal tract of mammals, frequently without causing any pathogenic effect on them [11]. Also, most strains of *E. coli* are not pathogenic to humans, but detection in any raw food intended for human consumption indicates a lack of hygiene during production, handling, processing or preparation Eventually, its detection in meat indicates fecal material contamination and the existence of many hazardous pathogens that could jeopardize the health and well-being of meat consumers [12]. Another important bacterial contaminant is *Shigella* which can result in illnesses with a low infective dose [13]. *Shigella* outbreaks of food poisoning are particularly common in hand-handled foods, such as butcher's meat, food subjected to limited heat treatment, or served undercooked [14].

Infections of *Salmonella* spp. are a leading cause of illnesses and death worldwide, particularly in poor nations where inadequate hygiene and lack of access to clean water and food are major problems [15]. The non-typhoidal *Salmonella* spp. is often related to gastroenteritis [16], while the typhoidal one manifest as enteric fever [17]. Around the world, the occurrence of *Salmonella* gastroenteritis is assessed at 93.8 million individuals with approx. 155,000 deaths [18].

The antibiotic resistance of *Salmonella* spp. remains an important concern of public health [19] especially in terms of influence on the effectiveness of treatment regimens and disease management [20]. In November 2016, a large typhoid fever outbreak started in Hyderabad city of Pakistan [21]. The outbreak first began in the city of Hyderabad and quickly spread to neighboring cities, including Jamshoro. Since August 2019, more than 10,000 XDR Typhoid cases have been reported from Hyderabad and Karachi alone [22].

In many Poultry slaughtering facilities of Hyderabad and Jamshoro continuous tap water supply is not available and butchers working in those slaughtering facilities are commonly using the stored water (collected from nearby homes or shops). Therefore, investigating microbial contamination on the hands of food handlers can go a long way in understanding the hygiene conditions of meat handlers (Fig. 1) and, as a consequence, in reducing foodborne illness.

In Hyderabad and Jamshoro districts of Pakistan, the status of butchers' hand hygiene and its connection to the microbial quality of water used for hand washing has not been studied previously. Hence, the aim of this study was the microbial analyses (*Salmonella, Shigella,* and *E. coli*) of the hands of the butchers and along with the quality of their hand washing water. Moreover, keeping in view the recent outbreak of typhoid the antibiotic resistance of *Salmonella* spp. is also determined against the commonly used antibiotics. Graphical abstract of the paper is shown in Fig. 1.

Methodology

Study area and sample size

The study was conducted in two selected districts of Sindh i.e. Hyderabad and Jamshoro (Fig. 2) and butchers working there were included in the study. Slaughtering facilities were selected from different areas of Hyderabad and Jamshoro such as, Jamshoro Phatak, Jamshoro phase 1, Naseem Nagar road, Qasimabad, Latifabad, and Hussainabad. Total 38 butcher shops were selected based on the convenient sampling technique and from each shop, two samples were collected, i.e. hand samples and water samples; hence, the study comprises of 76 samples out of which 38 were of hands' samples and 38 were of water samples. Out of 38 water samples 25, were from Hyderabad and remaining 13 were from the Jamshoro district.

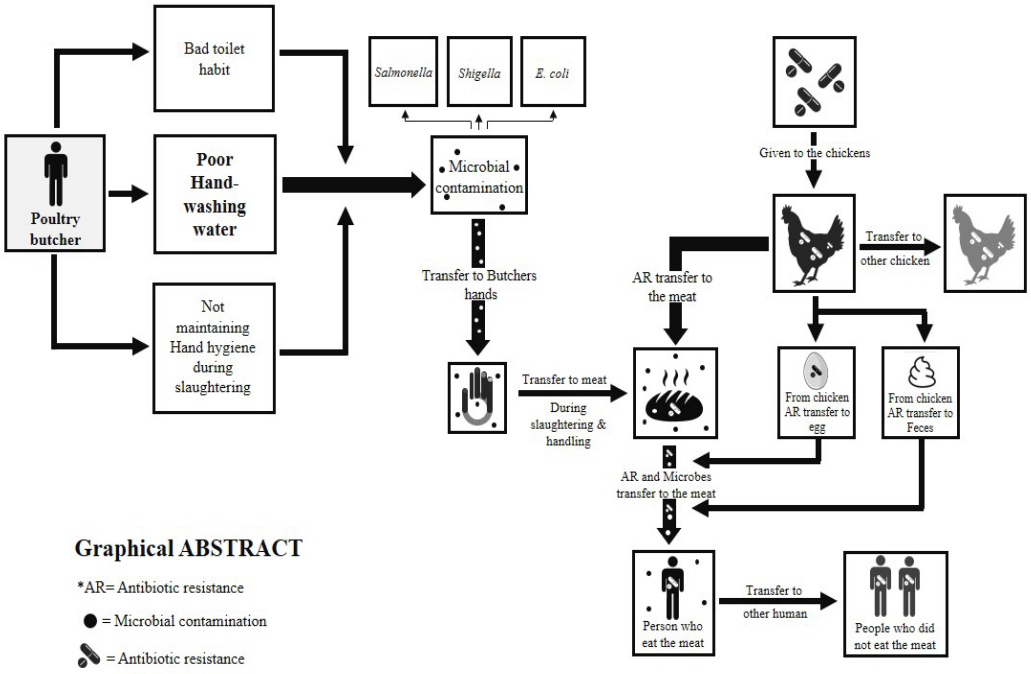

Fig. 1. Graphical abstract

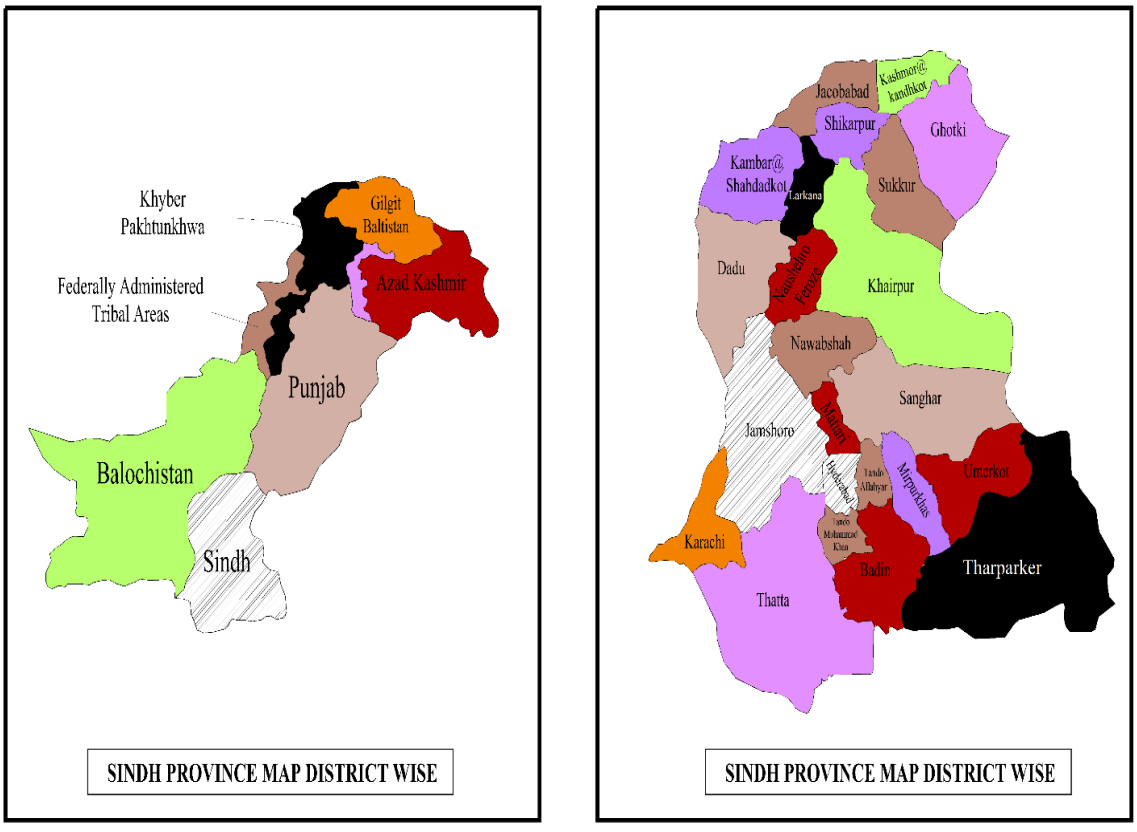

Fig. 2. Hyderabad and Jamshoro districts of Sindh Pakistan

Sample collection technique

Water used by poultry butchers for hand washing was collected from their containers by using a sterilized 15 mL Falcon tubes. While for hand hygiene assessment, samples were collected from their hands using sterilized swabs on 1 sq. inch approx. (shown in supplementary data Fig. S1). Swabs were then kept in the tubes containing 5 mL solution of 1-X Phosphate buffered saline (PBS) and were brought to the microbiology lab within 1 h.

Culturing, identification and enumeration of Salmonella, Shigella, and *E. coli*

For culturing of *Salmonella, Shigella,* and *E. coli,* Salmonella-Shigella (SS) agar (Fisher scientic, United States) was used and lab techniques were performed according to ISO 6579:2002 method [23]. Preparation of SS agar was carried out following the label instructions i.e. 60 g of agar powder was added in 1000 ml of distilled water. After that, the agar was boiled and poured into the agar plates. On solidification of agar, for hands samples, 100 µl of sample solution (sample mix with 1 X PBS) was inoculated on the agar plate and spread thoroughly with sterilized spreader. And for water samples 100 µl of sample water was directly inoculated on agar plates. Plates were then incubated for 48 h at 37°C. On completion of the incubation period, colonies of targeted bacteria were identified on the basis of their color difference (shown in supplementary data Fig. S2). The colonies on each plate were enumerated by using colony counter and were accepted in the range of 30–300 on each plate. The colonies of *Salmonella* spp. were sub-cultured on another SS-agar plate and were confirmed by using two biochemical tests, i.e. Triple Sugar Iron agar (TSI) and Urease test which are described in detail in below section.

Confirmatory tests

The confirmation of *Salmonella* spp. were done by using two biochemical tests, i.e. Triple sugar iron agar (TSI) tests and Urease test. For the performance of TSI agar test, the TSI agar was prepared as per label instruction and were poured in 20 ml falcon tubes. The tubes were kept in the slanted position and on solidification of agar, a deep slant is formed. After that a well-isolated colony of *Salmonella* from sub-culturing plate was inoculated in the agar by stabbing through the center and spreading on the surface of the agar. The tubes were then incubated for 24 h at 37°C. After incubation the colour of agar changed from dark pink to yellow and the bottom of the tubes bubbles were observed due to the formation of Hydrogen sulphide gas. It confirms the presence of *Salmonella* spp.

For urease test, the urea broth was prepared as per laboratory instruction and a loopful of pure *Salmonella* culture was inoculated with the help of sterilized loop. The tubes were then incubated for 3 to 4 days at 37°C. After completion of incubation time, the color of broth changed from yellow to pink due to the degradation of urea by S*almonella* and formation of ammonia gas.

Disc diffusion test

All the *Salmonella* isolates (24 from water samples and 33 from hand samples) were tested for their antimicrobial susceptibility by following the Kirby Bauer disc diffusion method. Total of nine antibiotics were tested and the selection of antibiotics was based on the common antibiotics used in Pakistan and those endorsed by the World health organization (WHO) for routine monitoring of antimicrobial resistance [22]. The antibiotics were identified as resistant, intermediate, and susceptible on the basis of their zones of inhibition [24]. For the performance of disc diffusion test, a loop full of presumptive *Salmonella* colonies were spread entirely over the Muller-Hinton agar (Sigma-Aldrich, United States) plates. Antibiotic discs, each containing a specific concentration of antibiotic such as, ampicillin (10 µg), erythromycin (15 µg), sulphamethoxazole (25 µg) azithromycin (15 µg), ceftazidime (30 µg), gentamicin (10 µg), streptomycin (10 µg) cefotaxime (30 µg), and neomycin (10 µg) were then applied. The plates were incubated for 24 h at 37°C. After completion of the incubation period the zones of inhibition around each antibiotic were measured in mm (shown in supplementary table S2 and S3) and were interpreted with the CLSI guidelines [24] (shown in supplementary data table S4). The isolates resistant to attest one antibiotic in three or more categories of antibiotics were considered MDR.

Statistical analyses

Laboratory results were recorded on each counting day. To determine the correlation between the microbial contamination of water samples and hand samples of butchers, the Pearson correlation was calculated by using Statistical Package for social sciences (SPSS). P value <0.05 was considered statistically significant.

Fig. S1. Swab sample collected from butcher's hand.

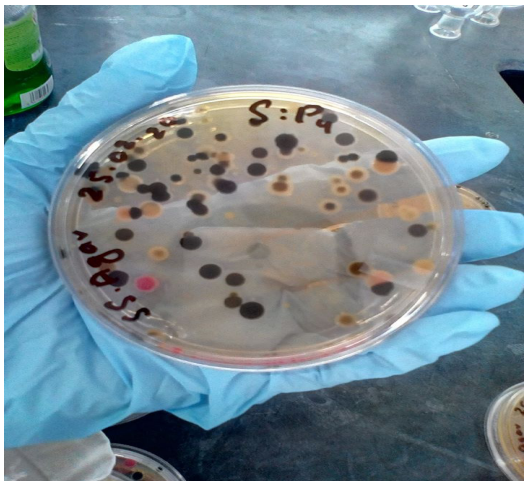

Fig. S2 . Culture plate showing the colonies of *Salmonella, Shigella* and *E. coli.*

Results

Microbial analyses of water and hand samples

The microbial assessment of water and hand samples revealed high prevalence of *Salmonella, Shigella,* and *E. coli*. In water samples prevalence of *Shigella* spp. and *E. coli* was found higher than *Salmonella* spp. and out of 38 samples, 30 (81.5%) of samples were positive for *Shigella* spp. *and E. coli* (each). However, the prevalence of *Salmonella* spp. was found as 29 (76.3%), shown in Fig. 3 (a).

The prevalence of *Salmonella, Shigella,* and *E. coli* in hands' samples is shown in Fig. 3 (b). It has been found out that out of 38 butchers' hand samples, 35 (92.1%), 36 (97.36%) and 38 (100%) of samples were positive for *Salmonella*, *Shigella* and *E. coli* respectively.

Enumeration of colonies isolated from water and butchers' hand samples

Table 1 and table 2 shows the number of samples fall in different cfu range (0, 1-300, 301-600, 601-900, 901-1200 and >1200). Out of 38 water samples 21.05%, 13.1%, and 18.42% of samples were having zero cfu/ml for *Salmonella, Shigella,* and *E. coli.* While 44.73%, 73.6% and 65.7% of samples were having more than 1200 cfu/ml for *Salmonella, Shigella,* and *E. coli.* For butchers' hands samples only three out of 38 (7.89%) and 1 out of 38 (2.63%) of samples were having zero colonies for *Salmonella* and *Shigella* while none of the samples were having zero colonies for *E. coli.* A large of samples showed more than 300 cfu/sq. inch for all three targeted bacteria.

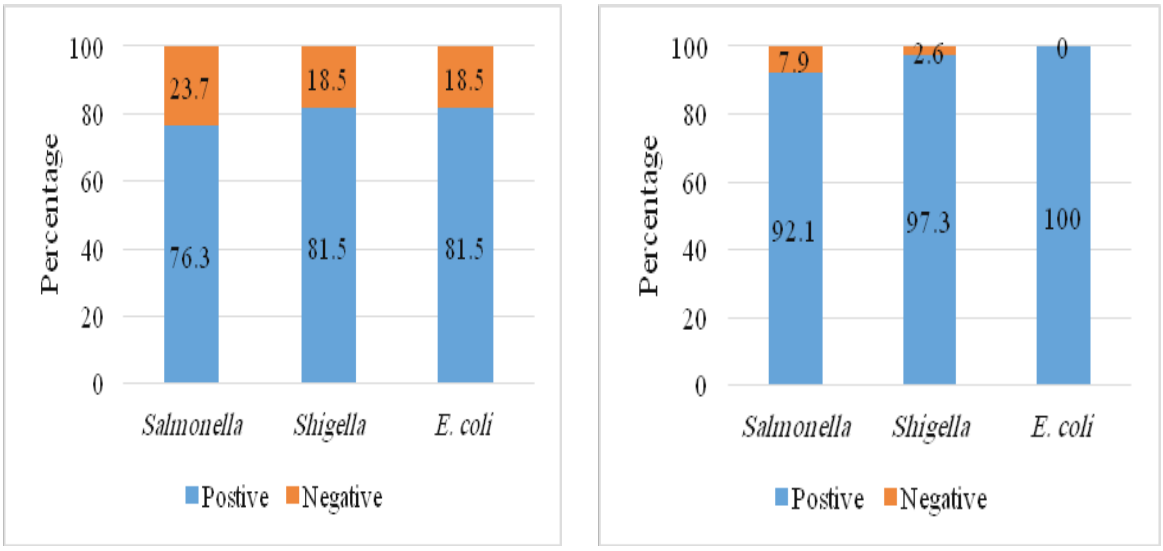

Fig. 3 (a). Prevalence of *Salmonella, Shigella* and *E. coli* in water samples (b) Prevalence of *Salmonella, Shigella* and *E. coli* in hand samples

TABLE 1. Colony forming unit (CFU)/ml of *Salmonella, Shigella*, and *E. coli* isolated from water samples

CFU Count range	No of samples *Salmonella* n (%)	No of samples *Shigella* n (%)	No of samples *E. coli* n (%)
0	8 (21.05)	5 (13.1)	7 (18.42)
1-300	4 (10.52)	2 (5.26)	3 (7.89)
301-600	2 (5.26)	0	1 (2.63)
601-900	4 (10.52)	2 (5.26)	3 (7.89)
901-1200	3 (7.89)	1 (2.63)	1 (2.63)
>1200	17 (44.73)	28 (73.6)	25 (65.7)
Total	38 (100)	38 (100)	38 (100)

n= Number of samples

TABLE 2. Colony forming unit (CFU)/ml of *Salmonella, Shigella,* and *E. coli* isolated from butchers' hands samples

CFU Count range	No of samples *Salmonella* n (%)	No of samples *Shigella* n (%)	No of samples *E. coli* n (%)
0	3 (7.89)	1 (2.63)	0
1-300	5 (13.15)	2 (13.15)	4 (10.52)
301-600	9 (23.68)	7 (18.42)	6 (15.78)
601-900	7 (18.42)	3 (7.89)	4 (10.52)
901-1200	9 (23.68)	8 (21.05)	10 (26.31)
>1200	5 (13.15)	17 (44.73)	14 (36.84)

n= Number of samples

TABLE S1. *Salmonella, Shigella* and *E. coli* colonies isolated from water and butchers' hands samples.

Sample ID	Salmonella (CFU/ml) water	Shigella (CFU/ml) water	E. coli (CFU/ml) Water	Sample ID	Salmonella (CFU/Sq. inch) Hand	Shigella (CFU/Sq. inch) Hand	E. coli (CFU/Sq. inch) Hand
W01	30	0	2000	H01	1050	1950	50
W02	0	10	0	H02	250	1100	10150
W03	0	1080	960	H03	1200	2250	1650
W04	0	2000	560	H04	400	1050	650
W05	2000	2000	0	H05	800	1000	950
W06	240	2000	2000	H06	150	550	3300
W07	50	180	290	H07	50	10000	550
W08	2000	2000	2000	H08	600	950	10000
W09	70	320	290	H09	640	8500	1100
W10	70	140	1300	H10	550	1240	500
W11	0	1280	1110	H11	0	1050	1450
W12	260	420	190	H12	100	500	950
W13	20	0	0	H13	150	1600	700
W14	170	230	110	H14	600	200	400
W15	0	250	0	H15	350	350	290
W16	0	30	80	H16	450	1500	2300
W17	90	80	0	H17	750	600	1450
W18	590	290	90	H18	3300	150	50
W19	730	590	390	H19	1600	900	1050
W20	0	0	0	H20	10000	10000	10000
W21	20	80	270	H21	1000	9400	450
W22	0	0	2000	H22	1500	10000	800
W23	260	270	0	H23	1050	1000	1650
W24	190	2000	130	H24	1500	2250	2150
W25	110	200	60	H25	450	1400	2450
W26	40	0	140	H26	600	950	1000
W27	20	220	70	H27	1150	800	1900
W28	140	320	180	H28	0	550	500
W29	190	180	220	H29	1050	0	1150
W30	220	2000	2000	H30	400	600	950
W31	120	210	130	H31	850	1950	4600
W32	180	330	130	H32	1100	2400	950
W33	200	670	30	H33	640	10000	2450
W34	90	220	290	H34	700	5550	50
W35	100	120	310	H35	950	3450	10150
W36	320	560	180	H36	0	1050	1650
W37	210	370	210	H37	1000	900	650
W38	110	160	340	H38	800	550	950

*W= Water samples, H=Hands samples.

TABLE S2. Zone of inhibitions (mm) of antibiotics for water samples:

Sample ID	Amp (10ug)	AZ (15 ug)	CAZ (30 ug)	CN (10 ug)	CTX (30 ug)	E (15 ug)	N (10 ug)	S (10ug)	STX (25 ug)
W01	0	26	19	11	24	0	14	18	26
W05	0	27	26	14	20	14	*0*	19	22
W06	0	23	26	17	22	16	15	29	0
W07	0	28	19	11	16	19	R	4	14
W08	0	20	25	23	30	0	23	3	0
W09	0	15	28	13	21	17	14	22	26
W10	0	17	20	19	10	18	30	7	5
W12	0	24	21	12	19	16	R	12	26
W13	5	17	20	33	22	8.5	18	3	6
W15	0	18	18	20	17	14	29	12	9
W18	0	23	28	7	9	11	16	5	23
W19	0	15	26	18	18	15	*0*	33	5
W20	0	20	19	14	31	0	21	6	0
W22	0	20	22	18	19	16	17	8	16
W24	19	22	27	14	17	19	17	10	14
W25	0	19	18	9	10	5	14	0	33
W26	0	26	25	28	15	17	18	38	21
W27	22	15	20	5	19	8	3.5	29	30
W28	2	20	19	21	16	8	24	21	10
W29	20	22	22	24	14	20	14	9	5
W30	0	16	32	19	24	12	18	8	0
W31	0	8	21	12	20	15	21	13.5	20
W32	0	27	26	22	17	18	14	34	22
W33	77	14	22	16	5	9	19	26	32
W34	4	17	29	27	20	4	17	21	26
W35	0	30	30	11	19	20	24	12	36
W36	0	28	33	24	16	6	27	13	R
W37	0	14	25	28	22	21	31	9	R
W38	0	14	38	31	10	17	19	4	4

* W= Water samples

* Amp= ampicillin, AZ= azithromycin, CAZ= Ceftazidime, CN= Gentamicin, CTX= Cefotaxime, E= E rythromycin, N= Neomycin, S= Streptomycin, STX= Sulphamethoxazole .

Antibiotic resistance profile of Salmonella

The antibiotic resistance profile of *Salmonella* spp. isolates from water and hands samples is shown in table 3, while the overall resistance pattern of isolates is shown in Fig. 4. In general, a high percentage of resistance to the tested antimicrobials was observed across all the isolates. For water samples, ampicillin showed the highest resistance profile, i.e. 89.6% of isolates were resistance. After ampicillin, streptomycin and sulphamethoxazole showed good resistance profile of 44.8% followed by erythromycin (41.3%) and gentamicin (24.1%). One isolate showed resistance for azithromycin. Cefotaxime and neomycin showed 23% and 17.2% of resistance. None of the isolates showed resistance for ceftazidime.

For butchers' hand samples, resistance profile of antibiotics was comparatively higher ($P > 0.01$). Out of 35 isolates, 88.5% of isolates showed resistance to ampicillin, 2.8% to azithromycin, 0% to ceftazidime, 28.5% to gentamicin, 28.5% to cefotaxime, 40% to erythromycin, 42.8% to neomycin, 28.5% to streptomycin, and 54.2% to sulphamethoxazole (shown in table 3).

TABLE 3. Resistance, intermediate, and susceptibility of *Salmonella* isolated from water and hands' samples against the antibiotics

Antibiotics	Water samples n (%)			Hand samples n (%)		
	R	I	S	R	I	S
Ampicillin (10 µg)	26 (89.6)	0	3 (10.3)	31 (88.5)	0	4 (11.4)
Azithromycin (15 µg)	1 (3.4)	10 (34.4)	18 (62)	1 (2.8)	12 (34.2)	22 (62.8)
Ceftazidime (30 µg)	0	8 (27.5)	21 (72.4)	0	10 (28.5)	25 (71.4)
Gentamicin (10 µg)	7 (24.1)	4 (13.7)	18 (62)	10 (28.5)	3 (8.5)	22 (62.8)
Cefotaxime (30 µg)	6 (23)	17 (58.6)	6 (23)	10 (28.5)	17 (48.5)	8 (22.8)
Erythromycin (15 µg)	12 (41.3)	17 (58.6)	0	14 (40)	21 (60)	0
Neomycin (10 µg)	5 (17.2)	14 (48.2)	10	15 (42.8)	9 (25.7)	11 (31.4)
Streptomycin (10 µg)	13 (44.8)	5 (17.2)	11 (37.9)	18 (51.4)	10 (28.5)	7 (20)
Sulphamethoxazole (25 µg)	13 (44.8)	3 (10.3)	13 (44.8)	19 (54.2)	3 (8.5)	13 (37.1)

R = Resistant; I = Intermediate; S=Susceptible
n= Number

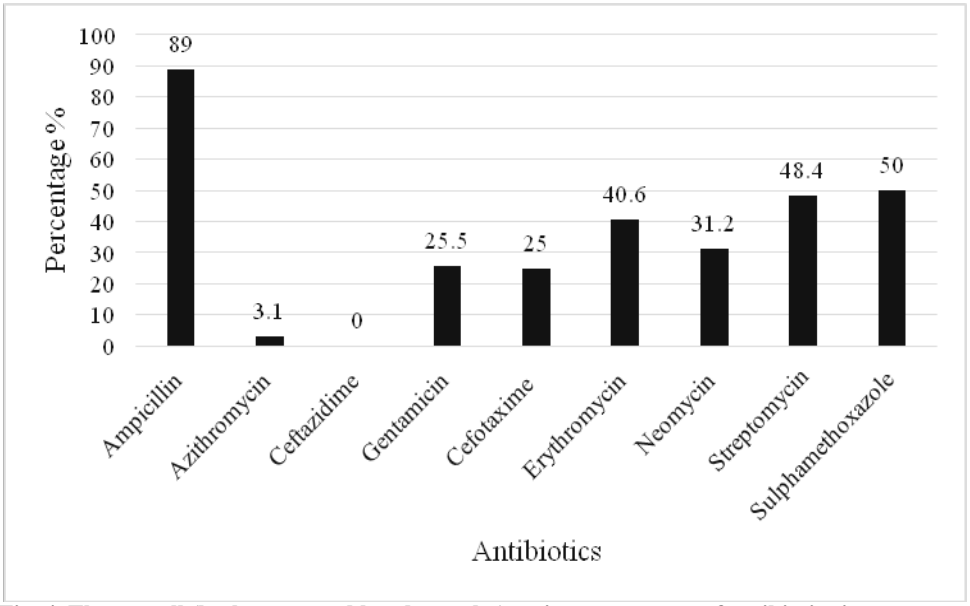

Fig. 4. The overall (both water and hand samples) resistance pattern of antibiotics in percentage.

TABLE S3 . Zone of inhibitions (mm) of antibiotics for hands Samples .

Sample ID	Amp (10ug)	AZ (15 ug)	CAZ (30 ug)	CN (10 ug)	CTX (30 ug)	E (15 ug)	N (10 ug)	S (10ug)	STX (25 ug)
H01	9	15	25	9	16	6	19	4	8
H02	14	28	28	33	0	8	20	8	26
H03	9	29	20	22	32	19	7	6	4
H04	11	16	21	19	17	10	22	14	8
H05	0	32	28	16	13	12	28	23	16
H06	0	35	19	27	18	20	0	26	23
H07	0	16	29	22	15	4	16	13	18.5
H08	0	25	32	11	12	16	30	7	7
H09	0	16	21	31	19	17	18	11	7
H10	16	20	35	11	18	9	0	4	11
H12	0	28	20	13	14	17	33	19	5
H13	0	15	37	16	20	16	17	17	6
H014	0	19	19	27	16	6	8	21	7
H15	0	33	32	12	19	19	14	28	0
H16	15	17	34	31	12	21	6	12	8
H17	0	16	29	28	38	0	21	6	26
H18	0	20	26	20	31	16	0	8	11
H19	0	23	22	10	26	16	29	13	19
H20	0	16	24	23	0	0	26	14	9
H21	9	21	20	29	16	17	13	12	22
H22	13	24	21	14	17	12	11	14	26
H23	10	16	30	8	4	18	12	9	22
H24	0	28	18	32	15	16	17	10	0
H25	16	30	28	13	14	19	8	0	5
H26	8	32	25	25	6	10	0	8	4
H27	10	30	19	6	19	17	15	11	8
H29	0	32	22	21	20	6	4.5	18	16
H30	0	19	22	15	34	16	18	6	25
H31	0	16	30	12	32	21	16	9	26
H32	9	16	25	18	7	8	5	21	23
H33	0	24	27	10	23	19	19	7	22
H34	0	13	20	25	29	10	0	6	14
H35	10	19	24	12	26	17	8	8	8
H37	0	15	29	31	0	19	19	19	25
H38	11	22	19	28	37	21	0	21	9
H01	9	15	25	9	16	6	19	4	8
H02	14	28	28	33	0	8	20	8	26
H03	9	29	20	22	32	19	7	6	4

* H= Butchers' hands samples.

Multi-drug resistant (MDR) Salmonella

In water samples 15 out of 30 (50%) and in butchers' hands samples 25 out of 35 (71.4%) of isolates were MDR (shown in Table 4). The overall percentage (including both water and hands sample isolates) of MDR isolates were 80%. The highest MDR phenotype observed were those resistant to four drugs (16% in water samples and 17.1% in hands samples). One XDR isolate was also found in one of the butcher's hands.

TABLE 4. Frequency and percentage of MDR isolates

Sample type	Frequency (n)	Percentage %
Water sample	15	50
Butchers' hands sample	25	71.4
Total	40	61.5

TABLE S4. CLSI Standards for antibiotic susceptibility testing

S#	Antibiotic Name	Code of disc	Potency	Resistant	Intermediate	susceptible
1	Ampicillin	Amp	10 µg	≤13	14-16	≥17
2	Azithromycin	AZ	15 µg	≤13	14-17	≥18
3	Ceftazidime	CAZ	30 µg	≤17	18-20	≥ 21
4	Gentamicin	CN	10 µg	≤12	13-14	≥ 15
5	Cefotaxime	CTX	30 µg	≤ 14	15--22	≥ 23
6	Erythromycin	E	15 µg	< 13	14-22	≥ 23
7	Neomycin	N	10 µg	≤13	13-18	≥19
8	Streptomycin	S	10 µg	≤11	12-14	≥15
9	Sulphamethoxazole	STX	25 µg	≤12	13-16	≥17

Discussion

The human body is a reservoir for many microorganisms. Microorganisms are transmitted to the hands during the process of food handling and through poor personal hygiene, which can lead to severe contamination of hands with many pathogens [25]. Meat handlers are an important vehicle for microorganisms and poor hand hygiene can contaminate meat and result in food-borne diseases that pose a potential health risk to the consumers [26]. Therefore, the present study was conducted to assess the prevalence of *Salmonella, Shigella,* and *E. coli* on the hands of the poultry butchers along with the quality of water which they are using for hand washing and rinsing the slaughtering tools. Similar bacterial contaminants have been reported by other authors in water, hands, food and environmental samples [27-29]. In the current study, all the collected water and hand samples were found heavily contaminated with *Salmonella Shigella,* and *E. coli.*

Because *E. coli* is commonly present in the intestines of humans and animals,[10], detection of this organism in butchers hands and water is generally considered an indicator of fecal contamination. Faecal contamination, in turn, indicates the presence of other harmful organisms, such as bacterial genera (*Campylobacter, Shigella*, and *Salmonella*), viruses (Rotavirus, Hepatitis A, Norovirus,) or parasites or protozoa (Giardia, Taenia, Toxoplasma, Cryptosporidium,) may also be there [30]. The poultry butchers can transfer these harmful organisms to the meat via handling and ultimately to the consumers. The contamination of *E. coli* is attributed to the poor water supply used during meat processing, feces of chickens, or from flies. The prevalence of *E. coli* in current study 81.5% (for water) and 100% (for hands samples) is very high than the similar kind of study conducted in Egypt where only 15% of the hand samples were contaminated with *E. coli* [31] and Nairobi, Kenya where prevalence of *E. coli* was found 78% [32].

In many developing countries Shigellosis is endemic and is responsible for causing at least 700,000 and 80 million bloody diarrhea cases [22]. Each year, large numbers of outbreaks of Shigellosis are caused by the consumption of contaminated foods [33]. Contamination with these organisms usually results from an infected person who uses improper techniques during handling or preparation [34]. The frequency of *Shigella* species and the prevalence of Shigellosis vary in different parts of the world.

In this study, *Shigella* spp. was isolated in 81.5% of the water samples and 97.3% of hand samples. The prevalence of *Shigella* spp. found in hands samples was higher as compare to water samples unlike the study conducted in Quetta, Pakistan in which only 16% of hand samples were contaminated with *Shigella* spp. [35]. According to the food and drug administration (FDA) less than 200 *Shigella* cells are enough to cause an infection, depending on the age and condition of the host (FDA, 2012). In the current study, more than 44.73% of water and 13.15% of hand samples were having > 1200 cfu/ml and cfu/sq. Inch, respectively.

Our findings for *Salmonella* spp. (76.3% from water and 92.1% from hands) aggress with some authors [36], who reported 91% isolation from abattoir worker's hands in Awka and in contrast with the study [37], who found 48.7% of *Salmonella* spp. isolation in Peshawar District of Pakistan. Contamination of Butchers' hands and their hand washing water with this pathogen is worrying; however, the situation is exacerbated if the contamination is with antibiotic resistant specie. The *Salmonella* spp. isolated in the present study were resistant to most of the tested antibiotics and the findings are in line with certain other studies conducted previously in poultry sector and slaughtering facilities of Pakistan [38-40].

The results revealed that in both water and hands' samples *Salmonella* spp. showed highest resistance to ampicillin and highest susceptibility to ceftazidime. Followed by ceftazidime, azithromycin is also found to be very effective with only one isolate from hands sample and one isolate from water samples being resistant to it. Similar kind of findings were obtained by Ramdhan et al. from Kohat Pakistan [41]. Thus, azithromycin and ceftazidime remain the only treatment options for *Salmonella* infections. Even though at a smaller rate, but the resistance of azithromycin against the isolates of *Salmonella* spp. will be an added threat. Preliminary studies of the potential treatment of SARS-CoV-2 with azithromycin [42] have resulted in irrational use of azithromycin even in patients with suspected COVID-19. Under the current scenario, unprecedented use of azithromycin in Pakistan (unpublished data) increases the likelihood of azithromycin being insensitive to *S. Typhi* XDR. If the use of azithromycin is not regulated, it is a ticking bomb in which typhoid fever will not be curable in Pakistan.

Gentamicin and erythromycin had showed good susceptibility in water samples but not in hands samples unlike the study conducted by Shaibu et al. where all the isolates of *Salmonella*, isolated from hands were susceptible to them [36]. The resistance percentage of streptomycin is in contrast to the study conducted in Southern Ethiopia where 97% of isolates showed resistance to streptomycin [43]. The high percentage of MDR *Salmonella* is in agreement with the study conducted by [44] and in contrast with the recent study conducted in Islamabad, Pakistan where 30% of *Salmonella* isolates were MDR [45].

This suggests possible transmission of antibiotic resistant bacteria through butchers' hands to poultry meat and from meat to the human body making the treatment of *Salmonella* infections difficult. To prevent such disease transmission hand washing is an established and effective way, [46] but this basic step which breaks the infection chain is not routinely performed by most of the meat handlers of Hyderabad and Jamshoro. In addition, with their bare hands, microorganisms can be transferred to the meat by sneezing or coughing as they handled the meat without using any protective clothing or gloves. For effective hand hygiene, a hygienic water source, typically potable water from a piped system or deep well is vital. With appropriate hygiene measures, the spread of infectious diseases can be reduced even in developing countries with limited resources [7]. Unfortunately, most of the slaughtering facilities were not having any visible source of running water and were making their own arrangements for water whose sources were not certain to acceptable limits. Storage of the water in dirty containers could also be a source of contamination as the butchers hardly cleaned the storage containers.

Recommendations

On the basis of the above discussion, the research suggests following recommendations 1) training on proper hand washing should be given to the butchers. 2) Use of gloves should be made necessary while working. 3) Quality of water used by poultry butchers should be improved and water should meet the potable water quality standards. In this regard, butchers should avoid the stored water if possible or maintain the cleanliness of storage containers on regular basis. 4) Establish hand-washing stations in poultry slaughtering facilities with hot water provision of minimum 112°F. Any hand washing material in the formed of soap or liquid hand wash should be available next to the tap every time. 5) Disposal towels for drying of hands should be available.

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Infectious and non-infectious diseases have become very common and endanger health [47-50] Medicinal plants and natural products can be used as preservatives for the hygiene of raw and processed foods or in the treatment of infectious and non-infectious diseases [51-53].

Conclusion

The study concludes the high presence of *Salmonella, Shigella,* and *E. coli* in poultry butchers hands as well as their hand washing water in two XDR Struck cities of Pakistan. The results of this study highlight the importance of meat handlers in transmitting pathogens to the consumers. The high microbial load in butchers' hands and their rinsing water is an indication of inadequate hygiene that can make the quality of meat handled by poultry butchers questionable and it may infect the consumers with many food borne infections. The presence of MDR *Salmonella* in such a high number and XDR (even though in small number) is alarming, with availability of limited treatment options. Because of high resistance of *Salmonella* isolates towards ampicillin and sulphamethoxazole, this study suggests that these drugs should not be used for the treatment of *Salmonella* infection.

Limitations of the study

The identification of species of *Salmonella Shigella,* and *E. coli* was not performed.

Similarly, serotyping for *Salmonella* was not performed due to financial issues.

Future recommendations

Although the study consists of small size of respondents, it gives an insight and direction of further studies on hand hygiene assessment. In future Species identification of *Salmonella, Shigella,* and *E. coli,* should be performed.

Abbreviations

CFU: Colony forming unit

CLSI: Clinical and Laboratory Standards Institute guidelines

FDA: Food and drug administration

MDR: Multiple drug resistant

PBS: Phosphate buffered saline

SPSS: Statistical package for social sciences

TSI: Triple-Sugar-Iron

WHO: World health organization

XDR: Extremely drug resistant

Conflict of interest

The author declared no conflicts of interest

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Authors contribution

Tagar, S. was responsible for data collection and analysis and writing of original draft and reviewing and editing were done by Ahmed, N.

References

1. Berhanu, L., Mereta, S.T., Gume, B., Kassa, T., Berihun, G., Dadi, L.S., Suleman, S., Tegegne, D., Getaneh, A. and Bedru, H. Effect of microbial quality of washing water on hand hygiene status of food handlers in Jimma town: implication for food hygiene and safety. *Journal of Multidisciplinary Health Care,* **14**, 1129-1134 (2021).

2. Park, H.-Y., Kim, S.K., Lim, Y.J., Kwak, S.H., Hong, M.J., Mun, H.M., Park, S.Y., Kim, H.J., Choi, H.R., Jeong, J.S. and Kim, M.N. Assessment of the appropriateness of hand surface coverage for health care workers according to World health Organization hand hygiene guidelines. *American Journal of Infection Control*, **42** (5), 559-561 (2014).

3. Stratev, D., Odeyemi, O.A., Pavlov, A., Kyuchukova, R., Fatehi, F. and Bamidele, F.A. Food safety knowledge and hygiene practices among veterinary medicine students at Trakia University, Bulgaria. *Journal of Infection and Public Health*, **10**(6), 778-782 (2017).

4. Assefa, T., Tasew, H., Wondafrash, B. and Beker, J. Community medicine & health education assessment of bacterial hand contamination and associated factors among food handlers working in the student cafeterias of Jimma. *Journal of Community Medicine and Health Education*, **5**(2), 1-8 (2015).

5. Nee, S.O. and Sani, N.A. Assessment of knowledge, attitudes and practices (KAP) among food handlers at residential colleges and canteen regarding food safety. *Sains Malaysiana,* **40**(4), 403-410 (2011).

6. Oranusi, S. U., Akande, V. A.and Dahunsi, S. O.. Assessment of microbial quality and antibacterial activity of commonly used hand washes. *Journal of Biological and Chemical Research*, **30**(2), 570-580 (2013).

7. Todd Ewen, C.D., Judy, D., Greig Barry, S., Michaels Charles, A., Bartleson Debra Smith, and John Holah. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 9. Washing and drying of hands to reduce microbial contamination. *Journal of Food Protection*, **73**(10), 1937-1955 (2010).

8. Ali, M.M., Verrill, L. and Zhang, Y. Self-reported hand washing behaviors and foodborne illness: a propensity score matching approach. *Journal of Food Protection*, **77**(3), 352-358 (2014).

9. Esterhuizen, L. Drinking water quality and farming practices on dairy farms in the greater Mangaung Metro, South Africa (Doctoral dissertation, Bloemfontein: Central University of Technology, Free State) (2004).

10. Lambrechts, A.A., Human, I.S., Doughari, J.H. and Lues, J.F.R. Bacterial contamination of the hands of food handlers as indicator of hand washing efficacy in some convenient food industries in South Africa. *Pakistan Journal of Medical Sciences*, **30**(4), 755 (2014).

11. Atnafie, B., Paulos, D., Abera, M., Tefera, G., Hailu, D., Kasaye, S. and Amenu, K. Occurrence of Escherichia coli O157: H7 in cattle feces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. *BMC Microbiology,* **17**(1) 1-7 (2017).

12. Zhang, W., Qi, W., Albert, T.J., Motiwala, A.S., Alland, D., Hyytia-Trees, E.K., Ribot, E.M., Fields, P.I., Whittam, T.S. and Swaminathan, B. Probing genomic diversity and evolution of Escherichia coli O157 by single nucleotide polymorphisms. *Genome Research*, **16**(6), 757-767 (2006).

13. Warren, B.R., Parish, M.E. and Schneider, K.R. Shigella as a foodborne pathogen and current methods for detection in food. *Critical Reviews in Food Science and Nutrition*, **46**(7), 551-567 (2006).

14. Garedew, L., Hagos, Z., Zegeye, B. and Addis, Z. The detection and antimicrobial susceptibility profile of Shigella isolates from meat and swab samples at butchers' shops in Gondar town, Northwest Ethiopia. *Journal of Infection and Public Health*, **9**(3), 348-355 (2016).

15. Ngogo, F.A., Abade, A.M., Rumisha, S.F., Mizinduko, M.M. and Majigo, M.V. Factors associated with Salmonella infection in patients with gastrointestinal complaints seeking health care at Regional Hospital in Southern Highland of Tanzania. *BMC Infectious Diseases*, **20**(1),1-8 (2020).

16. Smith, S.I., Seriki, A. and Ajayi, A. Typhoidal and non-typhoidal Salmonella infections in Africa. *European Journal of Clinical Microbiology & Infectious Diseases*, **35**(12), 1913-1922 (2016).

17. Mohan, A., Munusamy, C., Tan, Y.C., Muthuvelu, S., Hashim, R., Chien, S.L., Wong, M.K., Khairuddin, N.A., Podin, Y., Lau, P.S.T. and Ng, D.C.E. Invasive Salmonella infections among children in Bintulu, Sarawak, Malaysian Borneo: a 6-year retrospective review. *BMC Infectious Diseases*, **19**(1), 1-11 (2019).

18. Majowicz, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A., Hoekstra, R.M. and International Collaboration on Enteric Disease "Burden of Illness" Studies. The global burden of nontyphoidal Salmonella gastroenteritis. *Clinical Infectious Diseases,* **50**(6), 882-889 (2020).

19. Raji, M.A., Mamman, P.H. and Aluwong, T. Emerging strains and multidrugs resistant Salmonella species in humans and animals and the use of medicinal plants in Nigeria. *Global Research Journal of Microbiology*, **1**(1), 1-4 (2011).

20. Beshiru, A., Igbinosa, I.H. and Igbinosa, E.O. Prevalence of antimicrobial resistance and virulence gene elements of Salmonella serovars from ready-to-eat (RTE) shrimps. *Frontiers in Microbiology*, **10**, ID:1613-P:1-11(2019). doi: 10.3389/fmicb.2019.01613

21. Klemm, E.J., Shakoor, S., Page, A.J., Qamar, F.N., Judge, K., Saeed, D.K., Wong, V.K., Dallman, T.J., Nair, S., Baker, S. and Shaheen. Emergence of an extensively drug-resistant Salmonella enterica serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. *MBio*, **9**(1), 770-779 (2018).

22. World Health Organization. Shigellosis: disease burden, epidemiology and case management. *Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire*, **80**(11), 94-99 (2005).

23. ISO, 2007. Microbiology of food and animal feeding stuffs–Horizontal method for the detection of Salmonella spp.–Amendment 1: Annex D: Detection of Salmonella spp. in animal faeces and in environmental samples from the primary production stage. ISO 6579: 2002/Amd. 1: 2007.

24. Clinical and L.S. Institute, Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute Wayne, PA (2017).

25. Lues, J.F.R. and Van Tonder, I. The occurrence of indicator bacteria on hands and aprons of food handlers in the delicatessen sections of a retail group. *Food Control*, **18**(4), 326-332 (2007).

26. Campos, A.K.C., Cardonha, Â.M.S., Pinheiro, L.B.G., Ferreira, N.R., de Azevedo, P.R.M. and Stamford, T.L.M. Assessment of personal hygiene and practices of food handlers in municipal public schools of Natal, Brazil. *Food Control*, **20**(9), 807-810 (2009).

27. Okonko, I.O., Adejoye, O.D., Ogun, A.A., Ogunjobi, A.A., Nkang, A.O. and Adebayo-Tayo, B.C. Hazards analysis critical control points (HACCP) and microbiology qualities of sea-foods as affected by handlers hygience in Ibadan and Lagos, Nigeria. *African Journal of Food Science*, **3**(2), 011-022 (2009).

28. Sobukola, O.P., Awonorin, O.S., Idowu, A.M. and Bamiro, O.F. Microbial profile and critical control points during processing of 'robo'snack from melon seed (Citrullus lunatus thumb) in Abeokuta, Nigeria. *African Journal of Biotechnology*, **8**(10) 2385-2388 (2009).

29. Ukut, I.O., Okonko, I.O., Ikpoh, I.S., Nkang, A.O., Udeze, A.O., Babalola, T.A., Mejeha, O.K. and Fajobi, E.A. Assessment of bacteriological quality of fresh meats sold in Calabar metropolis, Nigeria. *Electronic Journal of Environmental, Agricultural & Food Chemistry*, **9**(1)89-100(2010).

30. Jay, James M., Martin J. Loessner, and David A. Golden. *Modern food microbiology*. Springer Science & Business Media, (2008).

31. Awadallah, M.A., Ahmed, H.A. and Merwad, A.M. Prevalence of non-O157 shiga toxin-producing Escherichia coli and Enterotoxigenic staphylococci in ready-to-eat meat products, handlers and consumers in Cairo, Egypt. **12**(5), 692-699 (2014).

32. Odwar, J.A., Kikuvi, G., Kariuki, J.N. and Kariuki, S. A cross-sectional study on the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya. *BMC Research Notes*, **7**(1),1-8 (2014).

33. Mead, P.S., Slutsker, L., Griffin, P.M. and Tauxe, R.V. Food-related illness and death in the United States reply to Dr. Hedberg. *Emerging Infectious Diseases*, **5**(6) **841**–842. PMCID: PMC2640798. (1999).

34. Lampel, K.A., Formal, S.B. and Maurelli, A.T. A brief history of Shigella. *EcoSal Plus*, **8**(1), 10.1128/ecosalplus.ESP-0006-2017. (2018). doi: 10.1128/ecosalplus.ESP-0006-2017

35. Saima, A.S., Ferhat Abbas, R., Rizwan, M., Yousaf, M., Hassan, Y., Naeem, M., Zahid, M., Pokryshko, O., Diaconescu, S. and Saifullah, S. Isolation & identification of Shigella species from food and water samples of Quetta, Pakistan. *Pure and Applied Biology (PAB)*, **7**(1), 227-235(2018).

36. Shaibu, A.O., Okolocha, E.C., Maikai, B.V. and Olufemi, O.T. Isolation and antibiogram of Salmonella species from slaughtered cattle and the processing environment in Abuja abattoirs, Nigeria. *Food Control*, **125**, 107972 (2021).

37. Rafiullah, A.A., Ali, M.I., Wazir, I., Khan, N., Shah, I.A., Khan, A. and Rashid, A.U. Antimicrobial resistance of salmonella species isolates from broiler birds in district peshawar. S. *Asian J. Life Sci*., **6**(2), 46-53 (2018).

38. Ali, R. and Saleem, S. Identification and quantification of antimicrobial activity in commercially available chicken meat in a large urban centre in Pakistan. *Current Research in Food Science*, **3**,173-177 (2020).

39. Koondhar, M.N., Kamboh, A.A., Khan, M.A., Leghari, R.A. and Dewani, P. Antimicrobial Resistance Profile of Salmonella spp. Isolated from Raw Beef Meat Samples Collected from Karachi, Pakistan. *Pakistan Journal of Zoology*, **53**(6) (2021).

40. Umair, M., Tahir, M.F., Ullah, R.W., Ali, J., Siddique, N., Rasheed, A., Akram, M., Zaheer, M.U. and Mohsin, M. Quantification and Trends of Antimicrobial Use in Commercial Broiler Chicken Production in Pakistan. *Antibiotics*, **10**(5), ID:598 (2021).

41. Ramadhan, A.H., Pembe, W.M., Omar, K.A., Xia, W. and Xu, Y. Characterization of antioxidant activity of peptide fractions from chinese giant salamander (*Andriaus davidianus*) protein hydrolysate. *J. Glob. Innov. Agri. Soc. Sci*., **5**, 14-19 (2017).

42. Zahid, I., Sarwar, A., Hussain, A., Sohail, M. and Amin, A. Antibiotyping and genotyping of extensively drug-resistant (XDR) Salmonella sp. isolated from clinical samples of Lahore, Pakistan. *Journal of Applied Microbiology*, **132**(1), 633-641 (2022).

43. Abdi, R.D., Mengstie, F., Beyi, A.F., Beyene, T., Waktole, H., Mammo, B., Ayana, D. and Abunna, F. Determination of the sources and antimicrobial resistance patterns of Salmonella isolated from the poultry industry in Southern Ethiopia. *BMC Infectious Diseases*, **17**(1), 1-12 (2017).

44. Elkenany, R.M., Eladl, A.H. and El-Shafei, R.A. Genetic characterisation of class 1 integrons among multidrug-resistant Salmonella serotypes in broiler chicken farms. *Journal of Global Antimicrobial Resistance*, **14**, 202-208 (2018).

45. Ali, A., Ali, H.A., Shah, F.H., Zahid, A., Aslam, H. and Javed, B. Pattern of antimicrobial drug resistance of Salmonella Typhi and Paratyphi A in a Teaching Hospital in Islamabad. *J. Pak. Med. Assoc.*, **67**(3), 375-379 (2017).

46. Siddiqui, T.R., Bibi, S., Mustufa, M.A., Ayaz, S.M. and Khan, A. High prevalence of typhoidal Salmonella enterica serovars excreting food handlers in Karachi-Pakistan: a probable factor for regional typhoid endemicity. *Journal of Health, Population and Nutrition*, **33**(1), 1-9 (2015).

47. Kaleemullah, M., Jiyauddin, K., Thiban, E., Rasha, S., Al-Dhalli, S., Budiasih, S., Gamal, O.E., Fadli, A. and Eddy, Y. Development and evaluation of Ketoprofen sustained release matrix tablet using *Hibiscus rosa*-sinensis leaves mucilage. *Saudi Pharmaceutical Journal*, **25**(5), 770-779 (2017).

48. Khan, J., Leenoos, L.M., Ruhi, S., Al-Dhalli, S., Kaleemullah, M., Saad, R., Ali, H.S., Sahu, R., Florence, M., Rasny, M. and Budiasih, S. Development and evaluation of polyherbal halal ointment using honey and Papaya. *International Journal of Medical Toxicology & Legal Medicine*, **23**(1and2), 232-238 (2020).

49. Ogwuegbu, M.C., Ani, A.O., Oyeagu, C.E., Edeh, H.O., Onodugo, M.O. and Ilo, S.U. Immunoglobulin and Oxidative Status Activities of Breast Meat in Broilers Fed Different Levels of Sodium Butyrate and Rosemary (*Rosmarinus officinalis* L) Leaf Meal. *Egyptian Journal of Veterinary Sciences*, **53**(2), 273-284 (2022).

50. Othman, Z., Khalep, H.R.H., Abidin, A.Z., Hassan, H. and Fattepur, S. The Anti-Angiogenic Properties of Morinda citrifolia. L (Mengkudu) Leaves using chicken chorioallantoic membrane (CAM) assay. *Pharmacognosy Journal*, **11**(1) 12-15(2019). DOI:10.5530/pj.2019.1.3

51. Mariam- Aisha, F. and Elisa, S.S. Optimisation of mint active compound extraction with anti-muscarinic property on intestine tissue. *International Journal of Medical Toxicology & Legal Medicine*, **22**(1and2), 200-203 (2019).

52. Wan, A.E., Khan, M.S.B., Teo, B.S.X., Khan, J., Abdullah, I., Kaleemullah, M., Asmani, F., Suofeiya, M., Al-Dhalli, S., Kasim, Z. and Fattepur, S. Screening of antioxidant and antibacterial activity of methanolic extract of Ipomoea aquatica leaf and stem against bacteria causes skin infection. *International Journal of Medical Toxicology & Legal Medicine*, **23**(3and4),169-178 (2020).

53. Zharif, N., Santosh, F., Kiran, C.N., Fadli, A., Ibrahim, A. and Nizam, G. Synergistic effect of ethanolic extract of melastoma malabataricum leaves and antibiotics. *International Journal of Medical Toxicology & Legal Medicine*, **21**(3and4),167-170 (2018).