Alfarama Journal of Basic & Applied Sciences

https://ajbas.journals.ekb.eg
ajbas@sci.psu.edu.eg

Faculty of Science Port Said University

http://sci.psu.edu.eg/en/

ISSN 2682-275X July 2022, Volume 3 Issue II DOI:10.21608/AJBAS.2022.122842.1090

Submitted: 05-03-2022
Accepted: 11-05-2022 Pages: 283-299

Antimicrobial Activity of Actinomycetes Extracts against Multidrug-Resistant *Staphylococcus aureus* and *Salmonella* spp. isolated from meat

Walaa Kamal Ahmed[1,*], Ihab Mahmoud Helal[2], Mahmoud Mohamed Zaky[1] Hesham Mohamed Abdulla[3]

[1]Botany Department-Faculty of Sciences Port-said University
[2]Animal Health Research Institution, ARC
[3]Botany & Microbiology Department – Faculty of Sciences Ismailia Suez Canal University
*** Corresponding author:** walaakamal30@yahoo.com

ABSTRACT

Foodborne diseases and poisoning are widespread in the world. It is a potential threat to human health. This study aimed to investigate alternative antibacterial compounds from actinomycetes isolated from medicinal plants in Sinai, Egypt, against multidrug-resistant foodborne microorganisms (*Salmonella* spp. and *Staphylococcus aureus)*. Bacteria are found in meat. A total of 100 randomly selected meat samples from the governorate of Port Said were obtained for this study. Out of those, 16% were found contaminated with *S. aureus*, while 7% were found contaminated with *Salmonella*. Susceptibility testing of the isolates to 12 antibiotics was performed using the Kirby-Bauer modified disc diffusion technique. All the *S. aureus* and *Salmonella* isolates were confirmed resistant to at least two antibiotics. About 100 % of *S .aureus* isolates were resistant to Ceftriaxone, 81.25% were resistant to Ampicillin, 75% were resistant to Oxytetracycline, while 100 % of *Salmonella* isolates were resistant to Erythromycin, and 85.7% were resistant to Rifampicin. Two of the *S. aureus* isolates showed multidrug-resistant to 6 antibiotics out of 12 antibiotics tested. One of the *Salmonella* isolates was also found resistant to 5 antibiotics out of 12 antibiotics tested. A total of 41 extracts from endophytic actinomycetes were screened for antibacterial activity against *S. aureus* and *Salmonella* which are multidrug- resistant. Out of 41 actinomycetes extracts 11 showed high antibacterial activity against the isolated foodborne pathogens (*S. aureus* and *Salmonella*). Results of the present study have shown that the antimicrobial compound derived from extracts of the endophytic actinomycetes may be useful in developing antibiotics against multidrug-resistant bacteria.

Key Words:

Actinomycetes, Fresh and Frozen meat, Multidrug-Resistant, *Salmonella* spp., *Staphylococcus aureus*.

1. INTRODUCTION

Food poisoning is a disease caused by consuming contaminated food. Human infection and food poisoning caused by food- borne pathogens have increased rapidly in Europe, the United States,

and other parts of the world over the past few years; meat and meat products are a major reported source of infection [1, 2]. Every year, millions of human cases are reported around the world, and the disease kills thousands of people even in the most developed countries [3].

Salmonella and *Staphylococcus aureus* are the most common bacteria found in animal-source foods among food-borne diseases. [4-8].

Globally, *Salmonella* accounts for over one-half of all food-borne diseases [9]. *Salmonella* is gram-negative rods belonging to Family Enterobacteriaceae that are mostly non-lactose fermenters, facultatively anaerobic, non-spore forming, mesophilic heterotrophs that produce acid and gas from glucose. The Kauffmann-White scheme 7, which includes nearly 2000 serotypes, is used to classify and identify them [10]. Salmonellosis is still one of the main sources of gastroenteritis in both people and animals. [11]. Antibiotic-resistant bacteria in food can pose a significant threat to health, resulting in infectious disease outbreaks in communities. Due to the rising prevalence of antibiotic resistance among *Salmonella* species, there is also the risk of therapeutic failure [12, 13]

The food-borne pathogen *Staphylococcus aureus* is very common. It is a highly adaptable human and animal microorganism that causes a wide range of illnesses, from minor skin infections to more serious illnesses including pneumonia and septicemia [14]. *Staphylococcus aureus* is a Gram-positive, nonmotile, round-shaped bacterium that does not produce spores. It's a facultative anaerobic bacterium that's commonly positive for catalase and nitrate reduction [15]. One of the most prevalent forms of food-borne illness is staphylococcal food poisoning (SFP) [16]. SFP has been described as the world's third leading cause of foodborne illness in recent decades [17]. The ingestion of one or more preformed staphylococcal enterotoxins (SEs) in contaminated food causes staphylococcal food poisoning (SFP). Because people can carry the microorganism, food contamination can occur as a result of infected food-producing animals or poor hygiene during the processing, transportation, and storage of foods. The number of *S. aureus* strains with antibiotic resistance has grown, as has the risk of infection of those properties by microflora through foods or producing difficult-to-treat illnesses [18]. Multiple antimicrobial resistance patterns have been observed in *S. aureus* [19].

Antimicrobial resistance is a significant public health issue in many countries, owing to the continual circulation of resistant strains of bacteria in the environment and the potential for food contamination [20]. The Multiple Antibiotic Resistance (MAR) index has been confirmed to be a dependable and cost-effective tool for detecting microorganisms. The MAR indexing method is low-cost, rapid, and easy to use. A MAR index value greater than 0.2 indicates a high-risk source of contamination where antibiotics are often utilized [21].

At the moment, most bacteria-caused diseases have developed resistance to most antibiotics [22]. Certain unfavorable side effects, as well as the growth of diseases linked to this novel antibiotic resistance, point to the necessity for the development of newer antimicrobial drugs capable of resisting both gram-positive and gram-negative bacteria [23-25]. Multi-drug resistance is currently a concern to patients in hospitals and communities, including widespread resistance to the first, second, and third generations of penicillins and cephalosporins [26, 27]. The rapid evolution of antibiotic resistance among microbial pathogens has an opportunity to explore actinomycetes as a source of novel antimicrobial drugs. In the twenty-first century, severe illnesses caused by bacteria resistant to regularly used antibiotics have become a serious global healthcare issue [22].

Actinomycetes are gram-positive bacteria that are aerobic, spore-forming, and have DNA with a high GC content (69-73%). They grow on a large branching substrate, aerial mycelia, and are

widely spread in the soil [28]. They've provided numerous key bioactive compounds with great economic value, and they're still being tested for novel bioactive compounds regularly. Actinomycetes have been found to contain around two-thirds of all naturally occurring antibiotics, including those with medicinal significance [29]. Actinomycetes, primarily the genera *Streptomyces* and *Micromonospora*, are known to produce about 80% of the world's antibiotics [30].

Endophytic bacteria that live inside medicinal plants make use of a unique environment (the plant's internal living tissues), which may allow them to produce bioactive substances that are comparable to those produced by their host. Endophytic actinomycetes have gotten a lot of attention in the search for new important bioactive substances that can be employed to develop new drugs to replace those that pathogenic strains have developed resistance to quickly [31]. The aim of this study is the screening the antimicrobial activity of some secondary metabolic products from actinomycetes extract as a natural source of an antimicrobial agent against 5 isolates of *S. aureus* and *Salmonella* spp. which are multidrug-resistant bacteria.

2. MATERIALS AND METHODS

2.1 Samples collection

A total number of 100 random samples of meat (40 fresh meat samples from butchers and 60 samples of frozen meat) were collected and transferred immediately under septic conditions by using sterile plastic bags. Samples were examined in Lab the laboratory of Animal Health Research Institute Port-Said from January 2015 till January 2016

2.2 Isolation methods

Isolation of *Staphylococcus aureus* and *Salmonella*, Samples (25 g/ml) were diluted with 225 ml of buffered peptone water (BPW) (Oxoid, Basingstoke, Hampshire, UK), homogenized in a stomacher and incubated at 37ºC for 16-20 h.

For *S. aureus* a loopful was taken and cultured onto Baird parker medium. All inoculated plates were incubated at (35+2 ºC) for 24-48h then colonies were identified. Suspected colonies of *S. aureus* were examined morphologically, microscopically [32] and biochemically [33] the purified colonies were analyzed via coagulase activity test by the inoculated fresh pure culture into sterile agglutination tubes containing 5 ml of brain heart infusion broth (B.H.I) and incubated at 37ºC Overnight and then 0.5 ml was transferred to tubes containing 0.5 ml of sterile citrated rabbit plasma. Inoculated tubes were incubated at 37ºC and examined for clot formation after 4 hours [34].

The procedures for isolation and identification of *Salmonella* spp. were conducted according to ISO 6579[35]. (0.1) ml of the incubated buffer peptone water was inoculated into sterile test tubes containing 10 ml (RVS broth) and incubated at 41.5 +1º C for 24 hr + 3 hr. A loopful of each RVS culture was streaked on to xylose lysine deoxycholate agar (XLD agar) (Difco, BD) Typical *Salmonella* colonies were selected from each specimen for confirmation based on biochemical characteristics using triple sugar iron agar test, lysine iron agar test, and Urease test [36]. Serotyping was performed using antisera (Difco,BD) in slide and tube agglutination tests based on the presence of the somatic O antigen and flagellar antigens according to the Kauffmann-White scheme[37]. *Salmonella* isolates were classified by Central Health Laboratories, Clinical Microbiology Unites in ElTahrir st, Dokki, Giza governorate.

2.3 Antimicrobial susceptibility testing

For the susceptibility test, pure cultures from selected isolates *S. aureus and Salmonella* were taken and transferred to a tube with 5 mL sterile saline solution and gently stirred to form a homogeneous suspension. With the sterile swab, the bacterial suspension was inoculated onto Muller–Hinton agar (Oxford, UK) to cover the whole surface of the agar. The plates were allowed to

dry at room temperature. The antimicrobial discs were kept at room temperature before being dispensed on the media's surface. The plates were then incubated for 24 hours at 37°C. Using calibrated rulers, the diameters of the inhibition zone of all around discs were determined to the nearest millimeters, and the isolates were categorized as susceptible, intermediate, or resistant according to CLSI recommendations [38]. Twelve antimicrobial agents in the form of disks were employed for susceptibility testing at the following concentrations: : Amoxicillin\Clavulanic Acid(AMC 30 µg),Ciprofloxacin (CIP 5 µg), Ampicillin AMP (10 µg) ,Doxycycline (DO 30 µg) ,Gentamicin (CN 10 µg),Oxytetracycline (OT 30 µg) ,Norfloxacin(NOR 10 µg) , Nalidixic acid (NA 30 µg) ,Ceftriaxone(CRO 30 µg) , Rifampicin (RD 5 µg), Erythromycin (E 15 µg) and Chloramphenicol (C 30 µg).

Multiple Antibiotic Resistance Index (MARI) Study:

The multiple antibiotic resistance index (MARI) of an isolate was equally derived using the mathematical expression of Blasco [39] which was given as:

MARI= a/b

Where (a) indicated the number of antibiotics to which the isolate was resistant, and (b) indicated the total number of antibiotics against which each isolate was tested.

Bacteria with a MAR index of >0.2 come from a high-risk source of contamination that uses several antibiotics or growth promoters, whereas bacteria with a MAR index < 0.2 come from a source that uses fewer antibiotics. The MAR index of a completely resistant isolate is 1.0

Identification of MDR (Multi-Drug Resistance)*:* According to krumperman [21] Resistance to two or more antibiotics among all antibiotics tested is referred to as multidrug resistance. The isolates' Multi-Drug Resistance (MDR) characteristics were analyzed to identify the isolates' resistance pattern to antibiotics.

٢.٤The endophytic actinomycete extracts

A total of٤١ endophytic actinomycete extracts, previously obtained from Sinai's wild medicinal plants [40] [41] were used for the antimicrobial screening against multiple antibiotic resistance *S. aureus* and *Salmonella.*

2.5. Organic metabolites extraction from actinomycetes:

The isolates' spore suspensions were cultured for 7 days at 28°C in 30 mL Starch Casein broth with 100 rpm shaking. Three times with ethyl acetate (1:1 v/v) and vigorous shaking for thirty minutes, the crude metabolites were extracted. Organic molecules were able to float in the less polar solvent as a result of this. For antimicrobial screens, the solvent layers were mixed and concentrated under vacuum using a rotary evaporator (HS2005S-N-Hahn Shin Scientific Co.); subsequently re-dissolved in ethyl acetate to give a final concentration of 100 µg/mL [42] with modification, for antimicrobial screenings.

2.6 Antimicrobial actinomycete extracts assay (Modified disc diffusion method, *NCCLS*, 2007):

The antibacterial activity of endophytic actinomycete extracts was evaluated using the disc diffusion method, which was modified from the National Committee for Clinical Laboratory Standards methodology. In a Muller Hinton agar, sterile antibiotic filter paper (Whatman, 6 mm) was impregnated with 10 µl and 50 µl of the reconstituted extract. Extracts were examined as duplicates. The plates were incubated for 18–24 hours at 37°C. The diameter of growth-free zones was used to calculate the diameter of inhibition zones.

3. RESULTS

3.1. Prevalence of *S. aureus* from examined samples of meat

Results of isolation and identification of *S. aureus* from 100 meat samples revealed that 13 isolates out of 40 fresh meat samples with percentage of 32.5% and from 60 frozen meat samples only 5 isolates with percentage of 8.3 % as shown in Table (1)

Table (1): Number and percentage of *S. aureus* positive samples from meat:

Types of samples	Number of examined samples	Bacteriological finding		Coagulase test			
		No. of positive samples	%	Positive		Negative	
				No.	%	No.	%
Fresh meat	40	13	32.5	11	84.6	2	15.3
Frozen meat	60	5	8.3	5	100	0	0
Total	**100**	**18**	**18**	**16**	**88.8**	**2**	**11.1**

S. aureus on Baird Parker media is characterized by circular, smooth, convex and black colonies with double layer hallow zone .The results of coagulase activity for 18 isolates of *S. aureus* were 16 isolates had a positive result with a percentage of (88.8)% while 2 isolates showed a negative result with a percentage of (11.1)%

Table (2): Number and percentage of *S. aureus* coagulase positive from meat:

Types of samples	Number of examined samples	No. of *S. aureus* coagulase positive	%
Fresh meat	**40**	**11**	**27.5%**
Frozen meat	**60**	**5**	**8.3%**
Total	**100**	**16**	**16%**

3.2. Prevalence of *Salmonella* from examined samples of meat

3.2.1. Results of isolation and biochemical identification of *Salmonella* from 100 meat samples revealed that 5 isolates out of 40 fresh meat samples with a percentage of 12.5% and from 60 frozen meat samples only 2 isolates with a percentage of 3.3 % as shown in Table (3)

Table (3): Number and percentage of *Salmonella* positive samples from meat:

Types of samples	Number of examined samples	Bacteriological finding	
		No. of positive samples	%
Fresh meat	40	5	12.5
Frozen meat	60	2	3.3
Total	**100**	**7**	**7**

Colonies of *Salmonella* were red-colored with black center on XLD agar On Triple sugar iron (TSI) agar test *Salmonella* spp. were alkaline red slant, acidic yellow butt with H_2S and gas production. On the lysine iron agar test *Salmonella* spp were alkaline purple butt and slant with H_2S production. on Urease test, Indole test, and Voges Proskauer test Salmonella sp. were negative results .on Methyl red test, Simmon's Citrate test *Salmonella sp*. were positive results.

3.2.2. Results of serological investigation and antigenic formula of Salmonella isolates

3.2.2.1. Autoagglutination test for serotyping

Salmonella is considered non-autoagglutination. one drop of saline solution was mixed with *Salmonella* colony on a clean glass slide using a loop, if the bacteria clumped into more or less distinct units, the strain was considered auto-agglutinable and not submitted to further serotyping procedures.

3.2.2.2antisera

Antisera One drop of polyvalent (O) antiserum was mixed with colony on a clean glass slide

Poly-O (A-E) antisera. Positive strains were further examined with individual O antisera (A, B, C, D, and E) to determine their group.

Following the identification of the isolated culture's group, the type was determined by identifying the (H) antigen, using polyvalent antisera containing both specific and non-specific H antisera. Using individual H antisera, the specific and non-specific phase of the strain was determined.

Table (4) antigenic formula of *Salmonella* isolates

Sample No.	Source	Organism	Antigenic formula
1	Fresh meat	*Salmonella* Entenitits 3	O:1,9,12 H_1:gm H_2:---
2	Fresh meat	*Salmonella* Entenitits 3	O:1,9,12 H_1:gm H_2:---
3	Fresh meat	*Salmonella* Entenitits 3	O:1,9,12 H_1:gm H_2:---
4	Fresh meat	*Salmonella* Anatum	O:3,(10),(15),(15,34) H_1:eh H_2:1,6
5	Fresh meat	*Salmonella* Anatum	O:3,(10),(15),(15,34) H_1:eh H_2:1,6
6	Frozen meat	*Salmonella* Muenster	O:3,(10),(15),(15,34) H_1:eh H_2:1,6
7	Frozen meat	*Salmonella* rough strain	----------

3.3 Result of Antibiotics sensitivity test

3.3.1. Result of sensitivity test of *S. aureus*:

Antibiotic sensitivity of 16 *S. aureus* isolates revealed that 16 (100 %) were resistant to Ceftriaxon, 13 (81.25%) were resistant to Ampicillin,12(75%) were resistant to Oxytetracycline, 9(56.2 %) were resistant to Nalidixic acid, 6 (37.5%) resistant to Erythromycin. on the other hand, 16(100%) were sensitive to Chloramphenicol , followed by15(93.7%) for Amoxicillin\Calvulanic Acid,14(87.5%) for each Doxycycline and Gentamicin , 13 (81.25%) for Norfloxacin, 11(68.7%) for Rifampicin and 10 (62.5%)for Erythromycin As shown on figure(1)

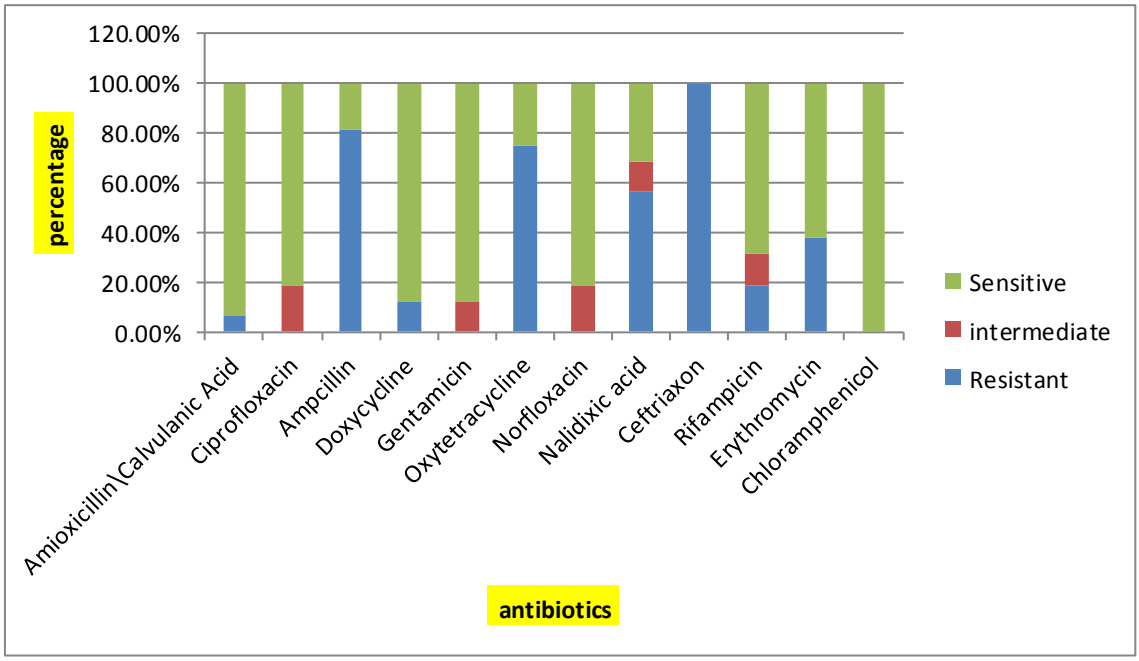

Figure (1) Antibiogram of *S. aureus*

Table (5) Multiple Antibiotic Resistance index of *S. aureus* isolates

Isolates no.	MARI=a/b
1	3/12=0.25
2	4/12=0.33
3	6/12=0.50
4	3/12=0.25
5	4/12=0.33
6	4/12=0.33
7	3/12=0.25
8	4/12=0.33
9	4/12=0.33
10	4/12=0.33
11	4/12=0.33
12	3/12=0.25
13	2/12=0.16
14	3/12=0.25
15	6/12=0.50
16	4/12=0.33

Table (6) MAR index values with Number of isolates

MARI	No. of isolates& %
0.16	1(6.25%)
0.25	5(31.25%)
0.33	8(50%)
0.50	2(12.5%)

All isolates of *S. aureus* represented resistance to at least two of the drugs assayed. This showed resistance against 2 to 6 antibiotics. The multiple antibiotic resistance Index calculated ranged from 0.16 to 0.50. As shown in table (5)

Two isolates of *S. aureus* were found to have the highest MAR index of 0.50 with a percentage (12.5%) which was resistant to 6 antibiotics from 12 antibiotics tested. Eight isolates of *S. aureus* were found with MARI equal to 0.33 with a percentage (50%) which were resistant to 4 antibiotics from 12 antibiotics .five isolates of *S. aureus* were resistant to 3 antibiotics from 12 and MRAI equal 0.25 with a percentage (31.25%). As shown in table (6)

3.3.2. Result of sensitivity test of *Salmonella*:

Antibiotic sensitivity of 7 *Salmonella* isolates revealed that 7 (100 %) were resistant to Erythromycin, 6 (85.7%) were resistant to Rifampicin, and 2(28.5%) were resistant to Nalidixic acid and Amoxicillin\Calvulanic Acid. on the other hand, 6(85.7%) were sensitive to Gentamicin and Oxytetracycline, and 5 (71.4%) were sensitive to Ciprofloxacin, Amoxicillin\Calvulanic Acid, Norfloxacin, Ampicillin, Chloramphenicol, and Doxycycline. As shown in figure (2)

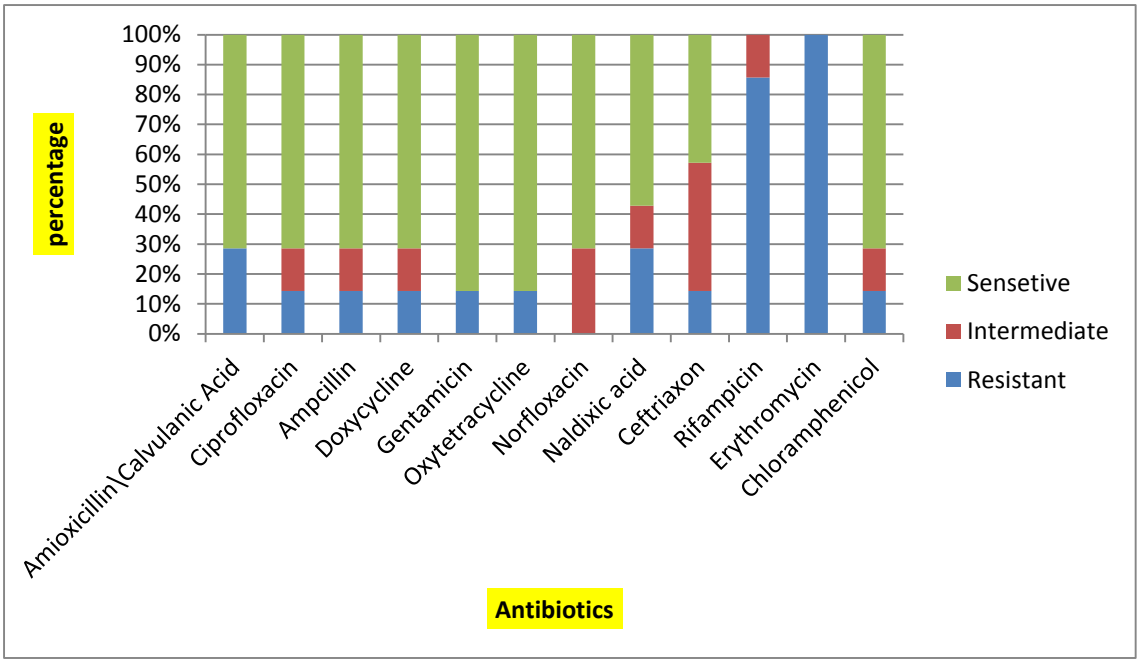

Figure (2) Antibiogram of *Salmonella*

Table (7) Multiple Antibiotic Resistance index of *Salmonella* spp isolates

Isolate no.	Serotype	MARI=a/b
1	*Salmonella* Entenitits 3	2/12=0.16
2	*Salmonella* Entenitits 3	4/12=0.33
3	*Salmonella* Entenitits 3	3/12=0.25
4	*Salmonella* Anatum	3/12=0.25
5	*Salmonella* Anatum	5/12=0.41
6	*Salmonella* Muenster	3/12=0.25
7	*Salmonella* rough strain	4/12=0.33

Table (8) MAR index values with the Number of isolates

MARI	No. of isolates& %
0.16	1(14.28%)
0.25	3(42.8%)
0.33	2(28.5%)
0.41	1(14.28%)

All isolates of *Salmonella spp.* represented resistance to at least two of the drugs assayed. This showed resistance against 2 to 5 antibiotics. The multiple antibiotic resistance Index calculated ranged from 0.16 to 0.41.as shown in table (7)

One isolate of *Salmonella* anatum was found to have the highest MAR index of 0.41 with a percentage (14.28%) which was resistant to 5 antibiotics from 12 antibiotics tested. Two isolates of *Salmonella*, *Salmonella* entenitits 3and *Salmonella* rough strain ,were found MARI equal to 0.33 with a percentage (28.5%) which were resistant to 4 antibiotics from 12 antibiotics .three isolates of *Salmonella* were resistant to 3 antibiotics from 12 and MRAI equal 0.25 with a percentage (42.8%). as shown in table (8)

3.4. Screening of actinomycete metabolites for antimicrobial activity against *Salmonella* and *S.aureus*

According to the results of the antibiotic susceptibility test, two isolates of *S.aureus* and three isolates of *Salmonella* which were the most resistant isolates to antibiotics were tested for antimicrobial activity of actinomycete metabolites.

The results of concentration of 10 µl of the extract were negative for all isolates, while the results of concentration of 50 µl showed that only 11 actinomycete extracts out of 41 actinomycete extracts exhibited antimicrobial activity against *S. aureus* and *Salmonella*.as shown in table (9)

Table (9) Zone of inhibition (mm) of actinomycetes antimicrobial activity

No. of antimicrobial agent	Isolates code	Plant source	Staph (1) Isolate no (3)	Staph (2) Isolate no (15)	Salmonella (1) Isolate no (2)	Salmonella (2) Isolate no (5)	Salmonella (3) Isolate no (7)
1	1/104 w2	*Lactuca orientalis*	8	10	14	10	7
2	2/34	*Mentha longifolia*	8	11	12	9	10
3	2/116	*Pulicaria undulata*	9	12	13	13	9
4	2/18	*Artemisia judicae L*	8	9	16	11	8
5	1/104 o-violet	*Lactuca orientalis*	10	15	14	12	9
6	1/104 w3	*Lactuca orientalis*	8	-ve	9	6	-ve
7	9/14	*Artimisia herba alba*	8	8	12	9	6
8	4/14	*Artimisia herba alba*	6	15	10	14	9
9	2/114	*Artemisia judicae L.*	10	10	12	9	7
10	10/14	*Artimisia herba alba*	-ve	-ve	12	17	12
11	7/105	*Malva parviflora*	-ve	7	11	15	8

Some of the actinomycete extracts showed broad-spectrum activity with wider zones of inhibition which are summarized in Table (10) and as show in (figure 3). According to these results two actinomycete isolates coded, 1/104 o-violet and 2/116 showed broad-spectrum antibacterial activity against most the bacterial isolates. Isolate code 2/114 is effective on *S. aureus* isolates while isolates code 10/14 and 7/105 are effective on *Salmonella*.

Table (10): Summary of the most active actinomycete extracts against *Salmonella* and *S. aureus*

S. aureus			*Salmonella*		
No. of antimicrobial agent	Isolates code	Zone of inhibition /mm	No. of antimicrobial agent	Isolates code	Zone of inhibition /mm
3	2/116	12	3	2/116	13
5	1/104 o-violet	15	5	1/104 o-violet	14
9	2/114	10	10	10/14	17
			11	7/105	15

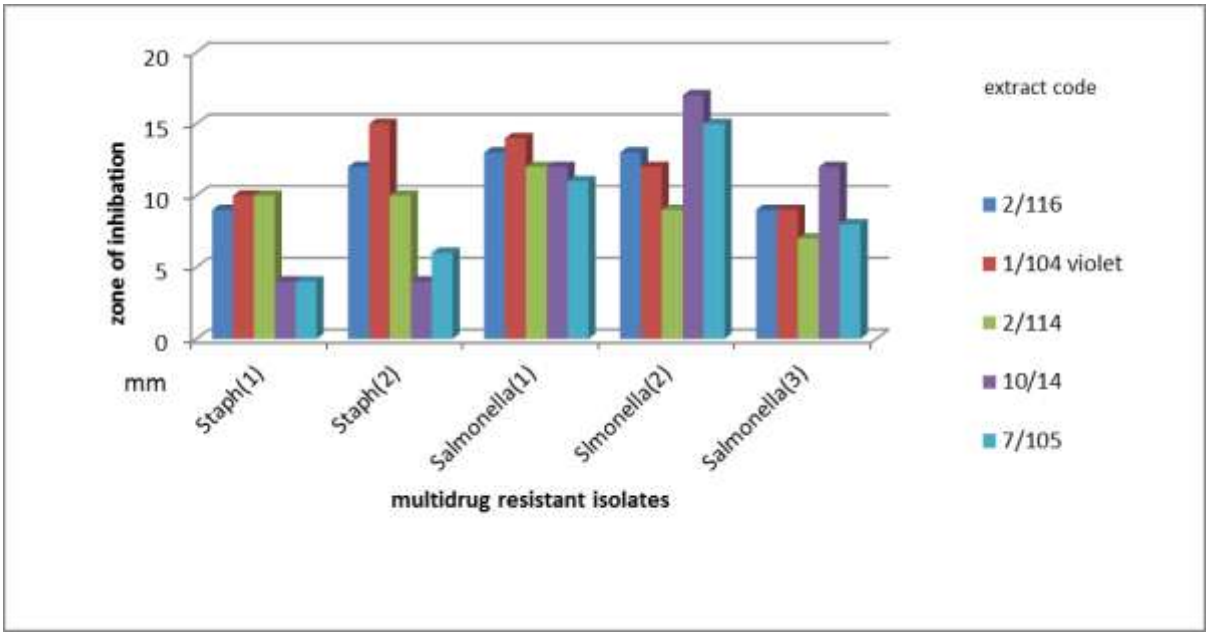

Fig (3) antibacterial effect of actinomycetes extract against multidrug-resistant isolates

4. DISCUSSION AND CONCLUSION

Pathogenic microorganisms are spread mostly through contaminated food. It is the leading cause of enteric diseases and the largest contributor to death and morbidity in impoverished nations [43]. Meats have a significant impact on food safety since they are a significant source of foodborne diseases. Food poisoning kills 420,000 people per year, with children under the age of five becoming especially susceptible, with 125, 000 children dying from foodborne infections each year according to World Health Organization (WHO) [44].

In the present study, a total of 100 samples of fresh and frozen meat were examined bacteriologically to reveal the prevalence of pathogenic *S. aureus* and *Salmonella* spp. at 16% and 7%, respectively. Different researchers all over the world confirmed the prevalence of pathogenic bacteria like *S. aureus* and *Salmonella* from food sources. This result agrees with the results obtained by [5] who reported that the prevalence of *S. aureus* and *Salmonella* spp was 18.18% and 5.25%

respectively isolated from raw meat in Thailand. On the other hand, our results were less than the result of [45] who reported the prevalence of *S. aureus* and *Salmonella* spp was 30% and 25% respectively isolated from fresh and frozen meat in Palestine. It's important to keep in mind that the main cause of staphylococcal food poisoning is staphylococcal enterotoxins (SEs). Because SEs toxins are extremely stable and heat resistant, reheating meat that contains SEs toxins produced by *S. aureus* at high temperatures may kill the bacteria but not the toxins. Consumers who are unaware of the properties of heat-stable toxins are putting their health at risk [46].

A food that is fully cooked can become re-contaminated if it touches other raw foods or drippings from raw foods that contain pathogens. The incidence of *S. aureus* and *Salmonella* in meat samples is an alarming figure and more attention is required in this respect. The high percentage of *S. aureus* and *Salmonella* spp in fresh meat more than in frozen meat is an indication of poor hygiene. Also, their contamination returned to the unhygienic manner, processing, transportation, storage and due to the insanitary condition of the butcher and absence of the health services in butcheries. Keeping frozen meat in sealed bags and not exposed to any contamination or air pollution made it less in the presence of pathogens

The results of antibiotics susceptibility revealed that, most of *S. aureus* strains isolated in this work were resistant to Ceftriaxone, followed by Ampicillin, Oxytetracycline, Nalidixic acid, Erythromycin, Rifampicin, Doxycycline and Amoxicillin\Calvulanic Acid . These results are in line with previous data [47], which recorded that *S. aureus* was resistant to tetracycline and ampicillin. [48], who reported that 60% of *S. aureus* isolates were resistant to ceftriaxone. Multidrug-resistant *S. aureus* has been reported several times [49]. Multidrug-resistance among foodborne bacteria has caused widespread worry owing to its public health and economic implications. For example, the US Centers for Disease Control and Prevention (CDC) reported that drug-resistant pathogens affect more than two million people in the US each year [50]. Additionally, 400000 people in Europe were estimated to be affected. The overuse of antibiotics in the medical field, including incorrect usage, lack of adherence to treatment standards, insufficient dose, and the use of therapeutic medicines as feed additives, is primarily responsible for the development of drug resistance among foodborne pathogens. In several countries, *S. aureus* has demonstrated great resistance to penicillin and other B-lactam antibiotics [51].

The MAR index is a helpful risk assessment tool, and its value (about 0.20) has been used to differentiate between low- and high-risk areas when antibiotics or growth promoters are overuse [52].

An analysis of this type displays the number of pathogens exhibiting antibiotic resistance in the susceptibility study's risk zone.

Multidrug-resistant *S. aureus* has become a major problem since the strains are often resistant to a wide range of antibiotics, including tetracyclines, aminoglycosides, macrolides, in addition to lincosamides. *S. aureus*, on the other hand, has a great genetic diversity, which complicates the development of control agents [53]. The multiple antibiotic resistance Index calculated ranged from (0.16 to 0.50) and for a total of 15 isolates from 16 isolates of *S .aureus* their MAR index was more than 0.25 indicating that these habitats had a significant level of antibiotic usage and selective pressure and a high-risk source of contamination where several antibiotics or growth promoters are used.

All *Salmonella* spp isolates were resistant to Erythromycin (100%), Rifampicin (85.7%), Nalidixic acid , Amioxicillin\Calvulanic Acid (28.5%), Gentamicin, Oxytetracycline, Ciprofloxacin, Norfloxacin ,Ampcillin, Chloramphenicol and Doxycycline(14.28%).

[54], reported that *Salmonella* resistant to rifampicin. [55], reported *Salmonella* isolates were resistant to erythromycin and tetracycline. [56], reported that all of the Salmonella isolates from meat were erythromycin resistant. On other hand [57], reported that *Salmonella* isolates showed resistance

to tetracycline, ciprofloxacin, and chloramphenicol. The high prevalence of resistance to these antimicrobial agents might be attributed to the uncontrolled use of antimicrobial agents as growth promoters or in the treatment of bacterial infections by farmers, who have unlimited access to these compounds and their usage [58]. Moreover, the results of this study agree with antibiotic resistance of *Salmonella* isolates from patients with diarrhea in Busan, South Korea [59]. These results indicate that meat might be one of the potential causes of antibiotic-resistant *Salmonella* human infections. The multiple antibiotic resistance

Index calculated ranged from (0.16 to 0.41) and a total of 5 isolates from 7 isolates of *Salmonella* spp their MAR index was more than 0.25

Numerous workers in India have reported multiple drug resistance to 6 to 8 antibiotics in several *Salmonella* serotypes of animal and human origin [60]. Multi-drug resistance is on the rise. *Salmonella* strains have been responsible for outbreaks and hyperendemicity of salmonellosis several times [61]. Antimicrobial resistance is generally encoded by plasmids in *Salmonella* strains, which have been acquired as a result of antibiotic pressure in humans and veterinary medicine. However, due to the fluidity of resistant plasmids and transposons, the antimicrobial drug resistance pattern cannot be recorded as a satisfactory method for discrimination within serovars.

The results of this study show that converting from routinely given antibiotics to treatments with higher potentiality for effective treatment and control of salmonellosis in humans is necessary and important.

Over the last 30 years, widespread antibiotic usage in animals has contributed to an increase in antibiotic resistance in many bacterial strains [62].

Salmonella and *S. aureus*, two foodborne infections, were found to be strongly linked to meat in this investigation. Foodborne pathogens that are resistant to antibiotics are a major public health concern. The presence of *Salmonella* and antibiotics appear to be ineffective against *S. aureus*, according to the study, poses a risk to consumer health. In terms of food safety, the development of antibiotic resistance to these common diseases is a cause for concern. The majority of regularly used *Salmonella* and *Staphylococcus aureus*. Antibiotic therapy is one of the most important therapies for infectious illnesses, and it has significantly improved public health. Nowadays, advancements in this treatment have resulted in the emergence and enhancement of drug-resistant pathogens, which can result in a variety of issues such as treatment failure, increased mortality and treatment costs, decreased infection control efficiency, and the spread of resistant pathogens from hospital to community. As a result, several studies have attempted to find new alternative methods to regulate and treat this problem [63].

Endophytic actinomycetes have been reported by certain researchers to have novel bioactive chemicals and enormous biological activity such as anti-bacterial, anti-fungal, anti-viral, anti-biofilm, anti-cancer, larvicidal, and so on[64,65] However, particularly in developing nations, simpler and more cost-effective local solutions, such as biocontrol agents, show enormous potential [66]. Traditional medicinal herbs, which have been investigated to some extent, might be used to produce biocontrol agents [67].

For the treatment of multidrug-resistant bacteria, new antibiotics must be discovered and developed. use of endophytic actinomycetes as antimicrobials, recovered from medicinal plants of Sinai are thought to be viable new sources for producing new bioactive compounds with antibacterial activity against the foodborne pathogens *Salmonella* and *S. aureus.* Actinobacteria extracts obtained from soil were found to be effective against a variety of pathogens, including *S. aureus* and *Salmonella* [68]. Antibacterial activities of herbs such as *Artemisia herb alba, Jasonia Montana*, and

Thymus vulgaris, as well as tree leaves of *Maesa lanceolata* and *Leucosidea sericea*, have been demonstrated against multidrug-resistant pathogens [69]. Endophytic actinomycetes appear to be a viable source of bioactive compounds that might be used for crop protection and medicinal drug development [70]. More than 140 actinomycetes genera have been identified as of now, although just a few of them are known to synthesize the most important antibiotics. Actinomycetes produce a variety of secondary metabolites, many of which have biological properties and might be used as therapeutics [71].

In our study a total of 41 selected extracts of the endophytic actinomycetes were used for this screening, the results showed that the ability of endophytic actinomycetes isolates to inhibit pathogenic bacteria varied and some of these extracts exhibited antimicrobial activity against *Salmonella* and *S.aureus* .The results of the screening showed broad-spectrum activity with wider zones of inhibition by using low concentaration 50 µl. According to these results and as shown in figure (3) two actinomycete isolates coded, 1/104 o-violet and 2/116, which were isolated from *Lactuca Orientalis* and *Pulicaria undulate* respectively, showed broad-spectrum antibacterial activity against most of the bacterial isolates. Isolate code 2/114,which was isolated from *Artemisia judicae L*., is effective on *S. aureus* isolates while isolates code 10/14 and 7/105, which are isolated from *Artemisia herba alba* and *Malva parviflora*, are effective on *Salmonella*. Previous studies have reported those endophytic actinomycetes extract has excellent antibacterial agent [72]. This result agrees with the results obtained by [56] who reported that 30 endophytic actinobacterial strains derived from medicinal plants found in the wild synthesize metabolic extracts, researchers employed three plants: *Mentha longifolia*, *Malva parviflora*, and *Pulicaria undulata*, together with their endophytic actinobacteria. Ethyl acetate was used to extract actinobacteria's crude metabolites. Multidrug resistant pathogens were inhibited by all metabolic extracts.

In this study, the antimicrobial compound produced from endophytic actinomycetes extracts was partially purified and its antimicrobial effects were studied. The crude culture supernatant had antibacterial activity against both Gram-positive and Gram-negative bacteria found in human foodborne diseases. When compared to commercial antibiotics, crude extract with the lowest concentration and purity of metabolites produced very effective results. These findings suggested that the compound we developed could be an alternative antibacterial agent that could be used to treat human diseases. Endophytic actinomycetes are a promising source of bioactive compounds and secondary metabolites for biotechnological use. Endophytic actinomycetes have created a number of new antibiotics that are effective against multidrug-resistant bacteria. Endophytes generate antimicrobial compounds that are environmentally friendly, hazardous to pathogens, and do not harm humans.

5. CONCLUSIONS

The findings from this study revealed the presence of *Salmonella* and *S. aureus* isolated from meat in Port-Said governorate. By screening the antibacterial activities of the crude metabolites of 41 different extracts of endophytic actinomycetes against foodborne pathogens isolated from meat and are multiresistant toward the commonly used antibiotics. The result indicated that some of these extracts exhibited varying antibacterial activity and the endophytic actinomycetes can be considered a potential novel source for new bioactive compounds with promising antimicrobial activity against bacteria pathogenic strains.

REFERENCES

[1] Angulo F , D Swerdlow(1999). AM Saeed, RK Gast, ME Potter, and PG Wall,eds .Iowa State University Press, Ames, IA, 33-41 , doi.org/10.1017/s0950268899233110.

[2] Abdellah, C., Fouzia, R. F., Abdelkader, C., Rachida, S. B. And Mouloud, Z (2008). Pak. J. Nutr,. **7**(2): 231-233, doi.org/10.3923/pjn.2008.231.233

[3] Bryan, F.L. and M.P. Doyle (1995) Journal of Food Protection,. **58**(3): 326-344 .doi.org/10.4315/0362-028x-58.3.326.

[4] Costa, L. F, Paixão, T A, Tsolis, R M, Bäumler, A J And Santos, R. L (2012) .Research in veterinary science, **93**(1): 1-6. Elsevier BV, https://doi.org/10.1016/j.rvsc.2012.03.002.

[5] Akbar, A and AKAnal (2013). Asian Pacific journal of tropical biomedicine. **3**(2): 163-168. https://doi.org/10.1016/s2221-1691(13)60043-x.

[6] Saba, C K. S., Escudero, J A, Herrera-Leon, S, Porrero, M C, Suarez, M, Dominguez, L, Demuyakor, B.And Gonzalez-Zorn, B,(2013).The Journal of Infection in Developing Countries, **7**(10): 691-695. https://doi.org/10.3855/jidc.3548.

[7] Moges Tiruneh, Mulat Dagnew (2013) .Journal of Infectious Diseases and Therapy, 1(2) :1-6. https://doi.org/10.4172/2332-0877.1000105.

[8] Mihaiu, L, Lapusan, A, Tanasuica, R, Sobolu, R, Mihaiu, R, Oniga, O And Mihaiu, M (2014) .The Journal of Infection in Developing Countries, 8(01): 050-058. https://doi.org/10.3855/jidc.3715.

[9] Curtiss III, R., S.M. Kelly, and J.O. Hassan(1993). Veterinary microbiology, **37**(3-4): 397-405. https://doi.org/10.1016/0378-1135(93)90038-9.

[10] Locke, S E, Kraus, L, Leserman, J, Hurst, M W, Heisel, S J And Williams, M R(1984). Psychosomatic Medicine,46(5):441-453. https://doi.org/10.1097/00006842-198409000-00005.

[11] Grimont, P.A. and F.-X. Weill (2007). WHO collaborating centre for reference and research on *Salmonella*, **9**: 1-166.doi.org/ 10.1111/j.1365-2672.2007.03368.x.

[12] Straley, B, Donaldson, S, Hedge, N, Sawant, A, Srinivasan, V, Oliver, S And Jayarao, B (2006) .Foodbourne Pathogens & Disease, **3**(3): 222-233.dio.org/ 10.1089/fpd.2006.3.222

[13] D White, S Zhao, R Sudler, S Ayers, S Friedman, S Chen, P McDermott, S McDermott, D Wagner, J Meng (2001).New England journal of medicine, **345**(16): 1147-1154.dio.org/ 10.1056/nejmoa010315.

[14] Lowy, F.D *(*1998). New England journal of medicine, **339**(8): 520-532.dio.org/ 10.1056/nejm199808203390806.

[15] M Masalha, I Borovok, R Schreiber, Y Aharonowitz, G Cohen (2001). Journal of bacteriology, **183**(24): 7260-7272 . dio.org/10.1128/jb.183.24.7260-7272.2001.

[16] Genigeorgis, C.A (1989). International journal of food microbiology, **9**(4): 327-360.dio.org/ 10.1016/0168-1605(89)90100-1.

[17] Zhang, S., J.J. Iandolo, and G.C. Stewart(1998). FEMS microbiology letters,**168**(2): 227-233. doi.org/10.1111/j.1574-6968.1998.tb13278.x.

[18] G Normanno, G La Salandra, A Dambrosio, N Quaglia, M Corrente, A Parisi, G Santagada, A Firinu, E Crisetti, G Celano(2007). International journal of food microbiology, **115**(3): 290-296. dio.org/10.1016/j.ijfoodmicro.2006.10.049.

[19] Enright, M.C.(2003). Current opinion in pharmacology, **3**(5): 474-479.dio.org/ 10.1016/s1471-4892(03)00109-7.

[20] Barber, DA, GY Miller, And PE Mcnamara (2003). Journal of food protection, **66**(4): 700-709.dio.org/ 10.4315/0362-028x-66.4.700.

[21] Krumperman, P.H(1983).Applied and environmental microbiology, **46**(1): 165-170.dio.org/ 10.1128/aem.46.1.165-170.1983.

[22] Alanis, A.J (2005).Archives of medical research, **36**(6): 697-705.dio.org/ 10.1016/j.arcmed.2005.06.009.

[23] Nathwani, D (2005) .International journal of antimicrobial agents, **25**(3): 185-192.dio.org/ 10.1016/j.ijantimicag.2004.11.006

[24] L Jevitt, A Smith, P Williams, P Raney, J McGowan, F Tenover,(2003) .Microbial Drug Resistance, **9**(4): 389-393.dio.org/ 10.1089/107662903322762833.

[25] Eliopoulos, G.M., V.G. Meka, and H.S. Gold (2004) .Clinical Infectious Diseases, **39**(7): 1010-1015.dio.org/ 10.1086/423841.

[26] Urban, C., S. Segal-Maurer, and J.J. Rahal (2003) .Clinical Infectious Diseases, **36**(10): 1268-1274.dio. 10.1086/374847.

[27] M Obritsch, D Fish, R MacLaren, R Jungv(2004) . Antimicrobial agents and chemotherapy, **48**(12): 4606-4610.dio. 10.1128/aac.48.12.4606-4610.2004.

[28] Oskay, A.M., T. Üsame, and A. Cem(2004). African journal of Biotechnology, 2 **3**(9): 441-446.dio. 10.5897/ajb2004.000-2087.

[29] Okami, Y. and K. Hotta, *editors, Goodfellow M., Williams ST and Mordarski M*. (1988).Academic Press Inc, New York, https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/referencespapers.aspx?referenceid=1954600

[30] Pandey, B., P. Ghimire, and V. Agrawal(2004).International Conference on the Great Himalayas: Climate, Health, Ecology, Management and Conservation, Kathmandu.. http://article.sapub.org/10.5923.j.microbiology.20180804.02.html

[31] Cao, L, Qiu, Z, Dai, X, Tan, H, Lin, Y And Zhou, S (2004) .World Journal of Microbiology and Biotechnology, **20**(5): p. 501-504.dio.org/ 10.1023/b:wibi.0000040406.30495.48

[32] Ryan, K.J. and C.G. Ray (2004). Sherris Medical Microbiology 4th edition McGraw Hill.

[33] FDA, F(2001). Guidance for industry: bioanalytical method validation. http://www. fda. gov/cder/Guidance/4252fnl. pdf.

[34] Collee, J.G., T.J. Mackie, and J.E. McCartney (1996). Harcourt Health Sciences,512-513.dio.org/ 10.1099/00222615-45-6-512b.

[35] Pavic, A., Groves, P., Bailey, G. And Cox, J. (2010) . Journal of Applied Microbiology, **109**(1): 25-34. dio.org/ 10.1111/j.1365-2672.2009.04649.x.

[36] Power, D.A. and J.A. Johnson (2009) . Manual of Microbiological Culture Media,92(2): 359-60.dio.org/ 10.1093/jaoac/92.2.459.

[37] Kauffmann, F. and W. Das-Kauffmann (2009). Acta Pathologica Microbiologica Scandinavica, 32(4) : 574-576 .dio.org/ 10.1111/j.1699-0463.1953.tb00291.x.

[38] Humphries, R, Bobenchik, A M, Hindler, J A and Schuetz, A N (2021). American Society For Microbiology, 59(12). https://doi.org/10.1128/jcm.00213-21.

[39] Schoeftner, S. and M.A. Blasco (2007).Nature cell biology, **10**(2): 228-236. dio.org/ 10.1038/ncb1685.

[40] El-Shatour, S, Abdulla, H, El-Karaaly, O, El-Kazzaz, W and Dewedar, A (2006) .Int J Bot, **2**(3): 307-312.dio.org/ 10.3923/ijb.2006.307.312.

[41] El-Shatoury, S ., El-Kraly, O, Trujillo, M, El-Kazzaz, W., Gamal El-Din, E and Dewedar, A (2013).Research in Microbiology, **164**(7): 761-769.dio.org/ 10.1016/j.resmic.2013.03.004.

[42] Forbes, B.A., D.F. Sahm, and A.S. Weissfeld (2016).Elsevier Health Sciences. dio.org/10.1093/clinchem/48.10.1816.

[43] Gunasegaran, T, Rathinam, X, Kasi, M, Sathasivam, K, Sreenivasan, S and Subramaniam, S (2011).Asian Pacific journal of tropical biomedicine, **1**(4): 266-269.dio.org/ 10.1016/s2221-1691(11)60040-3.

[44] World health organization https://www.who.int/news/item/03-12-2015-who-s-first-ever-global-estimates-of-foodborne-diseases-find-children-under-5-account-for-almost-one-third-of-deaths#

[45] Adwan, G., B. Alqarem, and K. Adwan(2015) International Food Research Journal, 22(5),1806-1812.

[46] Busani, L., Scavia, G., Luzzi, I., and Caprioli, A. (2006). Annali dell'Istituto superiore di sanitÃ,. **42**(4): 401- 404. PMID: 17361061.

[47] Heo JH, Lee ST, Chu K, Oh MJ, Park HJ, Shim JY, Kim M(2008). Korean Journal of Veterinary Research, **48**(1): 75-81. doi: 10.1111/j.1468-1331.2008.02157.x.

[48] Pickering, A, Hariri ,R, Harrison ,L,Marsh,J, Tasneem ,A,Freedy,H, Bonilla ,H(2014). Clinical Infectious Diseases, dio.org/ 10.1093/cid/ciu149

[49] Waters A, Contente-Cuomo T, Buchhagen J, Liu C, Watson L, Pearce K, Foster J, Bowers J, Driebe E, Engelthaler D, Keim P, PriceL(2001). Clinical Infectious Diseases, **52**(10): 1227-1230.dio.org/ 10.1093/cid/cir181.

[50] Centres for Disease Control and Prevention, US Department of Health and Human Services.: CDC; (2013): http: //www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

[51] Guven, K., Mutlu, M.B., Gulbandilar, A., Cakir, P., (2010). Journal of Food safety, 196–212. https://doi.org/10.1111/j.1745-4565.2009.00200.x

[52] Riaz, S., M. Faisal, and S. Hasnain.(2011). African Journal of Biotechnology. 10(33):6325–6331. DOI: 10.5897/AJB11.086

[53] Akindolire, M.A., Kumar, A., Ateba, C.N., (2018). Saudi Journal of Biological Sciences. 25(7), 1348–1355. https://doi.org/10.1016/j.sjbs.2015.10.011

[54] Björkman, J., D. Hughes, and D.I. Andersson. (1998).Proceedings of the National Academy of Sciences, **95**(7): 3949-3953. dio.org/10.1073/pnas.95.7.3949.

[55] Azage, M. and M. Kibret(2017). International journal of food science. dio.org/ 10.1155/2017/4317202

[56] Ameen F, Reda S, El-Shatoury S, Riad E, Enany M, Alarfaj A(2019).Saudi journal of biological sciences, 26(7): 1492-1498.dio.org/10.1016/j.sjbs.2019.09.008

[57] Ellerbroek L, Narapati D, Tai N, Poosaran N, Pinthong R, Sirimalaisuwan A, Tshering P, Fries R, Zessin K, Baumann M, Schroeter A(2010). Journal of Food Protection, **73**(2): 376-379.dio.org/ 10.4315/0362-028x-73.2.376.

[58] Hur J, J H Kim, J. H. Park, Y. J. Lee, and J. H. Lee. (2011). The Veterinary Journal, 189(3), 306-311. dio: 10.1016/j.tvjl.2010.07.017Cha, I.-H., Young-Hwan, B. Jae-Hun, and K. Kyoung-Soak. (1994).J. Korean Food Sci.Nutr. 23,927–932.

[59] Singh, S., Agarwal, R.K., Tiwari, S.C., Singh, H. (2012). Tropical animal health and production, 44(3): 665-74. https://doi.org/10.1007/s11250-011-9953-7

[60] Singh, B.R., Singh, M., Singh, P., Babu, N., Chandra, M., Agarwal, R.K. (2006). Journal of food protection, 69(2): 288-92. https://doi.org/10.4315/0362-028X-69.2.288

[61] Otalu O, Junaidu K, Chukwudi O, Jarlath U (2011). International Journal of Poultry Science,11(10),871-875.dio.org/ 10.3923/ijps.2011.871.875.

[62] Hashempour-Baltork F, Hosseini H, Shojaee-Aliabadi, S, Torbati, M, Alizadeh, A. M. and Alizadeh, M. (2019). Advanced Pharmaceutical Bulletin, vol 9, no. 3,335-347.doi: 10.15171/apb.2019.041

[63] Rajivgandhi G, Muneeswaran T, Maruthupandy M, Muthiah Ramakritinan C ,Saravanan K, Ravikumar V and Manoharana N. (2018). Microbial Pathogenesis, 125, 325-335. Dio:10.1016/j.micpath.2018.09.025

[64] Siddharth, S, Rai V R ,Ravishankar Rai,(2019) Microbial Pathogenesis, 137, 103775. https://doi.org/10.1016/j.micpath.2019.103775.

[65] Mushtaq, S., Shah, A.M., Shah, A., Lone, S.A., Hussain, A., Hassan, Q.P., Ali, M.N.,(2018). Microbial. Pathogenesis, 114, 357–361.Dio: 10.1016/j.micpath.2017.12.024

[66] Baskaran, S.A., Kazmer, G.W., Hinckley, L., Andrew, S.M., Venkitanarayanan, K., (2009) Journal of Dairy Science,92, 1423–1429.Dio:10.3168/jds.2008-1384

[67] Elbendary A, Hessain A, El-Hariri M, Seida A, Moussa I, Mubarak A, Kabli S, Hemeg H, El Jakee J (2018). Saudi journal of biological sciences, **25**(1): 44-46.dio.org/ 10.1016/j.sjbs.2017.05.003.

[68] Zeedan, G.S.G., Abdalhamed, A.M., Abdeen, E., Ottai, M.E., Abdel-Shafy, S., (2014). veterinary World. 7(11) .991-998. dio: 10.14202/vetworld.2014.991-998

[69] Pimentel-Elardo, S., Kozytska, S., Bugni, T., Ireland, C., Moll, H. and Hentschel, U., (2010). Marine Drugs, 8(2),.373-380. Dio:10.3390/md8020373

[70] Kekuda, T., (2016). International Journal of Current Microbiology and Applied Sciences, 5(7).100- 116.Dio:10.20546/ijcmas.2016.507.008

[71] Shaheen, N., N. Fatima, S. U. Sajid, and A. S. Gandapur. (2004 Journal of Ayub Medical College, Abbottabad: JAMC.16(4) .55–59