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Antibiotic Resistance in Staphylococcus aureus Isolated from Food Sources

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Abstract:Background: Staphylococcus aureus (S. aureus) is a major foodborne pathogen causing a spectrum of illnesses, ranging from mild gastroenteritis to life-threatening septicemia. The emergence of antibiotic-resistant S. aureus strains poses a significant public health concern. This study aims to investigate the prevalence and antibiotic resistance profiles of S. aureus isolated from various food sources within the food chain. Samples obtained from milk and meat samples in order to include any possible contamination sites. The isolates of S. aureus will be identified by means of recognized microbiological techniques. To identify patterns of resistance, antibiotic susceptibility testing will be carried out against a wide panel of antibiotics often used in both human and veterinary medicine. The results will clarify how antibiotic-resistant S. aureus is distributed throughout the food chain and how it can be involved in the formation and dissemination of antibiotic resistance. This data will be essential for developing methods to protect food safety and reduce the threat antibiotic-resistant S. aureus poses to public health

Keywords:*Staphylococcus aureus, foodborne pathogen, antibiotic resistance*

Introduction

Antibiotics are regarded as one of the twentieth century's most important discoveries, but due to the misuse and the overuse of antibiotics, antibiotic-resistant microorganisms rapidly appear **(Abd El-Aziz, El Sheikh et al. 2024).**

It is expected that by 2050, antimicrobial resistance (AMR) will cause millions of deaths worldwide due to decrease the effectiveness of antibiotics used nowadays and the absence of novel antibiotics in development **(de Kraker, Stewardson et al. 2016)**

One of these pathogenic resistant pathogen is*S. aureus*. It is a gram-positive bacterium that colonizes healthy humans and animals, and considered as an opportunistic pathogen when the immunity impaired **(Rungelrath and DeLeo 2021).**

S. aureus is categorized as a zoonotic pathogen of significant public health and veterinary importance **(Odetokun, Maurischat et al. 2022).** the presence of this pathogens in food products imposes potential

hazard for consumers and causes grave economic loss and loss in human productivity via food-borne disease (SFD). Symptoms of Staphylococcal food-borne disease include nausea, vomiting, and abdominal cramps with or without diarrhea **(Kadariya, Smith et al. 2014).**

It can contaminate food during preparation and processing due to staphylococcal enterotoxin (SE) production **(Kadariya, Smith et al. 2014).** Humans are susceptible to S. aureus infection from nosocomial infections in healthcare environments. Additionally, undercooked meat and unpasteurized milk are two examples of animal products that may contain it, and consuming these products could result in infection.

Nowadays, multi drug resistant (MDR) *S. aureus* were reported in food poisoning outbreaks **(Papadopoulos, Papadopoulos et al. 2018).** As Multi–drug resistant *S. aureus* isolated from milk, chicken meat, beef and egg **(Thaker, Brahmbhatt et al. 2013).**

The present study was carried out to isolate *Staphylococcus* spp. from various meat and milk samples and to determine their antibiogram.

Material and Methods

Isolation of s. aureus

Twenty s.aureus isolated from meat products and milk samples (n=10 each) on selective baired Parker Agar Base (HIMEDIA, M043-500G) which is selective and enriched media **(Chopin *et al.*, 1985)**. Also brain heart infusion (BHI) broth with 30% glycerol (Oxoid, UK) It was used for cryopreservation of bacterial isolates at -70ºC for future use.

Antimicrobial sensitivity test

The standard Kirby-Bauer disk diffusion method was used according to CLSI 2022 **(Lewis and James 2022)**. Müller Hinton agar (**CONDA, 1058.00)** plates were swabbed with Müller Hinton broth **(Oxoid, CM0405)** inoculated with the isolates (adjusted to match a McFarland obesity tube No. 0.5 by adding sterile saline). The antibiotic disks were placed on the inoculated plates, and then the plates were incubated at 37°C for 18-20 h. The plates were examined for the presence of the inhibition zone which indicated the susceptibility of the specific colonies to antibiotics. The zones of inhibition were then measured with a caliber and recorded.

1. **Antimicrobial discs**

All s. aureus isolates were tested against 12 antimicrobial discs of the following classes: vancomycin (VA, 30 µg), oxacillin(OX, 1 µg), ciprofloxacin (CIP, 5 µg), linezolid (LNZ, 30 µg), clindamycin(DA,2 µg), erythromycin (E,15µg), chloramphenicol(C,30 µg), cefuroxime (CXM, 30 µg), cefepime (FEP, 50 µg), ampicillin/sulbactam (SAM, 20 µg), gentamycin (CN, 10 µg), nitrofurantoin (F, 300 µg) **(table1).**

To confirm the resistance to vancomycin disc diffusion results, minimum inhibitory concentrations (MICs) were determined

D. Microdilution assay and interpretation of the result

One hundred microliters of the muller hinton broth were added to each well of the 96 well microtitre plates thendouble-fold serial dilutions with 100 µL of the antibacterial agent as vancomycin were made in custom-designed 96-well panels starting from a concentration of 1024 µg/mL.An equal volume of the prepared bacterial inoculum (100 µL) was added to all of the microtiter plates. Therefore, the initial concentrations of the antibacterial agents started with 512 µg/mL.Positive controls (wells containing inoculum but no antibacterial agent and negative controls (wells containing antibacterial agent but no inoculum) were applied. The microtiter plates were covered with the lid, sealed with tape and incubated at 37°C under aerobic conditions for 1-2 days. The first dilution with no microbial growth was recorded as MIC of the used agents. Subsequently, 10 µL aliquots from each well were plated onto MHA and the plates were incubated at 37ºC for 24 h to determine the MBC of the used agents.The plates were checked for the growth of bacterial colonies and MBC was evaluated as the lowest concentration of the antibacterial agents at which no growth was observed on the plates as MBC is defined as 99.99% decrease in CFU/mL **(Kwieciński, Eick et al. 2009).**

Half McFarland standard solution (McFarland 1907)

Half McFarland standard is a chemical solution of 50 µL of barium chloride (BaCl2, 1%) and 9.95 mL of sulfuric acid (H2SO4, 1%). The reaction between these two chemicals results in the production of fine precipitate of barium sulfate. It was used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of this McFarland standard, which is equivalent to a bacterial concentration of 1–1.5x10^{8} CFU/mL.

Table (1): Concentration and diameter of inhibition zones of antibiotics used for sensitivity test of *S. aureus* isolates

Antimicrobial class	Antimicrobial agent	Disc concentration	Diameter of inhibition zone		
			S	I	R
Beta-lactam	Oxacillin (OX)	1	≥18	_	≤17
Lincosamides	Clindamycin (DA)	2	≥21	15-20	≤14
Fluoroquinolones	Criprofloxacin (CIP)	5	≥21	16-20	≤15
Aminoglycosides	Gentamicin (CN)	10	≥15	13-14	≤12
Glucopeptides	Vancomycin (VA)	30	≤2	_	>2
Macrolides	Erythromycin (ERY)	15	≥23	14-22	≤13
Beta-lactam	Ampicillin/sulbactum (SAM)	20	≥16	15-17	≤18
Nitrofuran	Nitrofurantoin (F)	300	≥17	15-16	≤14
Cephalosporin	Cefuroxime(CXM)	30	≥19	15-18	≤14
Chloroamphinichol	Chloroamphinichol (C)	30	≥18	13-17	≤12
Cephalosporin	Cefepime (FEP)	50	≥24	_	<24
oxazolidinones	Linezolid (LNZ)	30	≥21	_	≤20

***The interpretation of inhibition zone was according to (CLSI, 2022)**

Determination of MDR and MAR index among S. aureus isolates

Multidrug-resistant bacteria (MDR) is a synonym designated for isolates found resistant to at least one agent in three or more antimicrobial classes. The multiple antibiotic resistance (MAR) index was determined using the previously described formula: a/b, where a indicates the number of antibiotics to which the isolate exhibits resistance, while b represents the total number of the tested antimicrobials to which the isolate was exposed. Isolates with a MAR index exceeding 0.2 come from a high-risk contamination source that uses various antibiotics, whereas bacteria with a MAR index of less than 0.2 come from a source that uses fewer antibiotics. The MAR index of a fully resistant isolate is 1**(Blasco, Esteve et al. 2008).**

Result

Fig (1): s. aureus on baired parker media appear as black colony with hallow zone

Antimicrobial sensitivity testing

As showen in table (2), *S. aureus* isolates showed (100%) resistance to oxicillin, ampicillin/sulbactum, cefuroxime, cefepime, and vancomycin, followed by (85%) resistance to ciprofloxacin, and erythromycin, (80%) to gentamycin and nitrofurantoin, (55%) to chloramphenicol , (30%) clindamycin and (10%) linezolid.The isolates were VRSA based on disc diffusion sensitivity test and MIC values obtained from broth microdilution test.

Detection of MDR and MAR index for S. aureus isolates

All *S. aureus* isolates were MDRaccording to the antibiotic sensitivity test, and their MAR index was ≥ 0.2 ranged from (0.5 to 0.91) as showen in **table(2).**

Table (2): Antibiotic resistance patterns of *S. aureus* isolated from different sources and their MAR index

Isolates number	Isolate source	Resistance profile	MAR index
***S* 1**	**Food**	**OX, VA, E, FEB, SAM, CXM, F**	0.58
***S* 2**	**Food**	**OX, VA, E, FEB, SAM, CXM**	0,5
***S* 3**	**Food**	**OX, VA, CIP, E, FEB, SAM, CXM**	0.58
***S* 4**	**Food**	**OX, VA, E, FEB, SAM, CXM**	0.5
***S* 5**	**Food**	**OX, VA, CIP, E, FEB, SAM, CN, CXM**	0.66
***S* 6**	**Food**	**OX, VA, CIP, E, FEB, SAM, CN, CXM, F**	0.75
***S* 7**	**Food**	**OX, VA, CIP, E, FEB, SAM, CN, CXM, F,**	0.75
***S* 8**	**Food**	**OX, VA, CIP, FEB, SAM, CN, CXM, F, DA**	0.75
***S* 9**	**Food**	**OX, VA, CIP, FEB, C, SAM, CN, CXM, F**	0.75
***S* 10**	**Food**	**OX, VA, CIP, FEB, C, SAM, CN, CXM, F**	0.75
***S* 11**	**Milk**	**OX, VA, CIP, E, FEB, C, SAM, CN, CXM, F, DA**	0.91
***S* 12**	**Milk**	**OX, VA, CIP, E, FEB, C, SAM, CN, CXM, F, DA**	0.91
***S* 13**	**Milk**	**OX, VA, CIP, E, FEB,C, SAM, CN, CXM, F, , DA**	0.91
***S* 14**	**Milk**	**OX, VA, CIP, E, FEB, SAM, CN, CXM, F, DA**	0.83
***S* 15**	**Milk**	**OX, VA, CIP, E, FEB , C, SAM, CN, CXM, F,**	0.83
***S* 16**	**Milk**	**OX, VA, CIP, E, FEB, C, SAM, CN, CXM, F,**	0.83
***S* 17**	**Milk**	**OX, VA, CIP,E, FEB, C, SAM, CN, CXM, F, LNZ**	0.91
***S* 18**	**Milk**	**OX, VA, CIP, E, FEB, C, SAM, CN, CXM, F, DA**	0.91
***S* 19**	**Milk**	**OX, VA, CIP, E, FEB, C, SAM, CN, CXM, F, LNZ**	0.91
***S* 20**	**Milk**	**OX, VA, CIP, E, FEB C, SAM, CN, CXM, F, DA**	0.91

Discussion

Food-borne diseases (FBD) are a major public health concern worldwide which defined by World Health Organisation (WHO) as "disease of infectious or toxic nature caused by, the consumption of food or water **(Le Loir, Baron et al. 2003).** Staphylococcal Food-Borne Disease (SFD) is one of the most common causes of

reported FBD in the United States **(Murray 2005).** Symptoms of SFD include hypersalivation, nausea, vomiting, and abdominal cramping with or without diarrhea. If significant fluid is lost, physical examination may reveal signs of dehydration and hypotension **(Kadariya, Smith et al. 2014).**

The present study desingned to detect the presence of s.aurous meat and milk samples

In this study all samples contaminated with s.aurous which indicate that food exposed to unhygenic conditions, as the prevalence of s.aurous related to the personal hygiene and food handler according to **(Ghosh, Wahi et al. 2007).**

The primary route of spreading resistant microorganism and resistance genes from food animals to humans are food products of animal origin that are tainted with resistant bacteria. Direct interaction with animals or an environment where animals are present **(Casey, Curriero et al. 2013).** Food can become contaminated with *S. aureus* from the food-producing animals or from infected food handlers during various processing stages **(Al-Amery, Elhariri et al. 2019).**

The isolates of *S. aureus* with reduced susceptibility to vancomycin are classified into three groups by the Clinical and Laboratory Standards Institute. These are vancomycin-susceptible *S. aureus* (VSSA) with MIC ≤ 2 μg/ml, vancomycin-intermediate *S. aureus* (VISA) with MIC of 4–8 μg/ml, and VRSA with MIC ≥ 16 μg/ml. **(Werner, Strommenger et al. 2008),** in this study all isolates from meat and milk were VRSA

In this study the majority of the isolates from meat and milk demonstrate resistance to oxicillin, ampicillin/sulbactum, cefuroxime, cefepime, and vancomycinand this agree with **(Goswami, Trivedi et al. 2011)**as it warned from the increase of infections caused by antibiotic-resistant bacteria, particularly in the emergence of VRSA/VISA.

The majority of the isolates about 90% show sensitivity to lenzolid and according to this study as it prove that linezolid resistance occurs in **≤1% of S**. aureus isolates **(Stefani, Bongiorno et al. 2010)**

When bacteria is 'resistant to three or more antimicrobial classes' it is called MDR. it is based on *in vitro* antimicrobial susceptibility test results, when they test 'resistant to multiple antimicrobial agents, classes or subclasses of antimicrobial agents' **(Cohen, Calfee et al. 2008).** Here, all the isolates are multi drug resistant (MDR). The high frequency of MDR isolates has been related to the excessive use and improper use of antibiotics, including in livestock husbandry, self-medication, and substandard infection control and prevention practices **(Salam, Al-Amin et al. 2023).**

Conclusion:

This study revealed that milk and meat samples were contaminated with MDR and MAR *Staphylococcus* isolates, and having potential public health significance. It is necessary to Apply precautionary measures to avoid the contamination

References:

Abd El-Aziz, E., S. El Sheikh, A. Galal and Y. Refky (2024). "Possible Alternative Strategies to Combat Antimicrobial Resistance." Zagazig Veterinary Journal 52(1): 1-24.

Al-Amery, K., M. Elhariri, A. Elsayed, G. El-Moghazy, R. Elhelw, H. El-Mahallawy, M. El Hariri and D. Hamza (2019). "Vancomycin-resistant Staphylococcus aureus isolated from camel meat and slaughterhouse workers in Egypt." Antimicrobial Resistance & Infection Control 8: 1-8.

Blasco, M., C. Esteve and E. Alcaide (2008). "Multiresistant waterborne pathogens isolated from water reservoirs and cooling systems." Journal of applied microbiology 105(2): 469-475.

Casey, J. A., F. C. Curriero, S. E. Cosgrove, K. E. Nachman and B. S. Schwartz (2013). "High-density livestock operations, crop field application of manure, and risk of community-associated methicillin-resistant Staphylococcus aureus infection in Pennsylvania." JAMA Intern Med 173(21): 1980-1990.

Cohen, A. L., D. Calfee, S. K. Fridkin, S. S. Huang, J. A. Jernigan, E. Lautenbach, S. Oriola, K. M. Ramsey, C. D. Salgado and R. A. Weinstein (2008). "Recommendations for metrics for multidrug-resistant organisms in healthcare settings: SHEA/HICPAC position paper." Infection Control & Hospital Epidemiology 29(10): 901-913.

de Kraker, M. E., A. J. Stewardson and S. Harbarth (2016). "Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050?" PLoS Med 13(11): e1002184.

Ghosh, M., S. Wahi, M. Kumar and A. Ganguli (2007). "Prevalence of enterotoxigenic Staphylococcus aureus and Shigella spp. in some raw street vended Indian foods." Int J Environ Health Res 17(2): 151-156.

Goswami, N. N., H. R. Trivedi, A. P. Goswami, T. K. Patel and C. B. Tripathi (2011). "Antibiotic sensitivity profile of bacterial pathogens in postoperative wound infections at a tertiary care hospital in Gujarat, India." J Pharmacol Pharmacother 2(3): 158-164.

Kadariya, J., T. C. Smith and D. Thapaliya (2014). "Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health." Biomed Res Int 2014: 827965.

Kwieciński, J., S. Eick and K. Wójcik (2009). "Effects of tea tree (Melaleuca alternifolia) oil on Staphylococcus aureus in biofilms and stationary growth phase." International Journal of Antimicrobial Agents 33(4): 343-347.

Le Loir, Y., F. Baron and M. Gautier (2003). "[i] Staphylococcus aureus [/i] and food poisoning." Genetics and molecular research: GMR 2(1): 63-76.

Lewis, I. and S. James (2022). "Performance standards for antimicrobial susceptibility testing." (No Title).

McFarland, J. (1907). "The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines." Journal of the American Medical Association 49(14): 1176-1178.

Murray, R. (2005). "Recognition and management of Staphylococcus aureus toxin-mediated disease." Internal medicine journal 35: S106-119.

Odetokun, I. A., S. Maurischat, V. O. Adetunji and A. Fetsch (2022). "Methicillin-Resistant Staphylococcus aureus from Municipal Abattoirs in Nigeria: Showing Highly Similar Clones and Possible Transmission from Slaughter Animals to Humans." Foodborne Pathog Dis 19(1): 56-61.

Papadopoulos, P., T. Papadopoulos, A. S. Angelidis, E. Boukouvala, A. Zdragas, A. Papa, C. Hadjichristodoulou and D. Sergelidis (2018). "Prevalence of Staphylococcus aureus and of methicillin-resistant S. aureus (MRSA) along the production chain of dairy products in north-western Greece." Food microbiology 69: 43-50.

Rungelrath, V. and F. R. DeLeo (2021). "Staphylococcus aureus, Antibiotic Resistance, and the Interaction with Human Neutrophils." Antioxid Redox Signal 34(6): 452-470.

Salam, M. A., M. Y. Al-Amin, M. T. Salam, J. S. Pawar, N. Akhter, A. A. Rabaan and M. A. A. Alqumber (2023). "Antimicrobial Resistance: A Growing Serious Threat for Global Public Health." Healthcare (Basel) 11(13).

Stefani, S., D. Bongiorno, G. Mongelli and F. Campanile (2010). "Linezolid Resistance in Staphylococci." Pharmaceuticals (Basel) 3(7): 1988-2006.

Thaker, H. C., M. N. Brahmbhatt and J. B. Nayak (2013). "Isolation and identification of Staphylococcus aureus from milk and milk products and their drug resistance patterns in Anand, Gujarat." Veterinary World 5: 10.

Werner, G., B. Strommenger and W. Witte (2008). "Acquired vancomycin resistance in clinically relevant pathogens."