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***Serratia marcescens* isolated from neonate patients with septicemia and study of the inhibition effect of Garlic plant extracts compared to antimicrobial agents in Thi Qar province in Iraq**

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Abstract--Detection of Extended spectrum beta lactamase (ESBL) enzyme producing bacteria in hospital settings is vital. This study was carried out to determine the distribution of *Serratia marcescens* that producing ESBL isolated from neonate patients from Al-Imam Al-Hussein hospital at Thi-qar province during a period of nine months from May to December 2020. Out of 100 swabs and samples collected from blood specimens of infant patients, bacterial growth was identified in 22 cultures (22%). The results showed that after treating 22 *Serratia* isolates with antibiotics, 22 isolates were identified as *Serratia* producing ESBLs and resistant 100% to antibiotics after incubating the plates cultured with *Serratia* bacteria for a whole isolates. During this study, garlic extract was used to compare the effect of the extract with isolates that showed high resistance. Where the results showed that there were inhibition rates after treating 22 isolates of *Serratia* with garlic extract (hexane, alcohol, hexane and alcohol mixtures), where 65% of the samples showed inhibition in different proportions, and 35% showed resistance and inhibition percentages appeared only with alcohol and as for hexane and hexane and alcohol mixtures. No results appeared isolates.

Keywords--isolated, patients, septicemia, antimicrobial.

Introduction

Serratia marcescens is a gram-negative, aerobic, motile bacteria that is commonly said to as the *Serratia* genus' most prevalent primary pathogenic species. Although it is common in the environment, it is not a common component in human feces (Mandell et al., 2009; González-Juarbe et al., 2015). It can be distinguished from other bacteria because it produces a characteristic red coloring. Prodigiosin (Elahian et al., 2013; Andreyeva and Ogorodnikova, 2015). Non-pigmented strains of *S. marcescens* are more resistant to antibiotics than pigmented strains due to the presence of resistant plasmids (Auwaerter, 2007). *Serratia marcescens* may have multidrug-resistant mechanisms, such as the development of beta-lactamase enzymes, which makes therapy more difficult (Daoudi et al., 2018; Cristina et al., 2019). Multidrug resistance, particularly resistance to extended-spectrum beta-lactamase among *Serratia* species, has become a global problem for human medicine. As a result, research were conducted to conduct epidemiological investigations and to identify these resistance enzymes at a molecular level (Elsherbiny et al., 2018; Simsek, 2019).

Serratia marcescens is the most pathogenic species in the genus *Serratia*. (Merkier et al., 2013), Wound infection, urinary and respiratory tract infections, pneumonia, sinusitis, septic arthritis, meningitis, and septicemia are all disorders that can be caused by this bacteria (Mostatabi et al., 2013; Mohajerani et al., 2019). In neonatal intensive care units (NICUs), *Serratia marcescens* causes sepsis, pneumonia, urinary tract infections, and conjunctivitis, but it is a rather uncommon pathogen (Jones et al., 2000; Stoll et al., 2002; Ulu-Kilic et al., 2013) The urinary tract was one of the most important isolation sources for *S. marcescens*, with infection closely linked to urologic interventions such cystoscopy, transurethral surgery, contamination of urine containers and urination sites, and especially catheterization (Buffet-Bataillon et al., 2009). *Serratia* has also been linked to more dangerous diseases such as cellulitis, which is an inflammation of the subcutaneous connective tissue (Pithadia et al., 2019).

Garlic, or *Allium sativum*, is a plant that belongs to the Lilliacease family and is native to Central Asia. It may now be found all over the world. Various species of garlic have been used as a spice or condiment to flavor food for centuries. Garlic has been used in herbal medicine to treat various diseases (Azeez et al., 2015). This plant is thought to help with blood sugar regulation and cardiovascular health. It is also antibacterial, anticarcinogenic, antioxidant, and anti-inflammatory (Iwalokun et al., 2004). Furthermore, some studies have found that garlic has antibacterial properties against various types of bacteria (Azeez et al., 2015) . Immediately after a wound, whether caused by a severe burn, trauma, or surgery, the affected area is at risk of colonization by a variety of microorganisms, which proliferate within the damaged tissues and cause infection (Rafla, and Tredget, 2011) .

Materials and Methods

Sample collection and bacterial identification

During a period of nine months from May to December 2020, Twenty two *S. marcescens* isolates were collected from Al-Imam Al-Hussein hospital at Thi-qar province isolates were isolated mostly from blood specimens of infant patients. Initial identification of *S. marcescens* isolates based on morphological characteristics of the colonies, such as colony shape, colony texture, and color, was studied in relation to bacterial growth on MacConkey agar, whereas initial identification of *S. marcescens* isolates based on morphological characteristics of the colonies color was studied in relation to bacterial growth on CHROM agar orientated. Isolates of *Serratia marcescens* were identified at the species level using the VITEK® 2 compact system and Identification gram-negative bacteria (ID-GNB) cards, as directed by the manufacturer.

Collection and preparation of plant extracts

Garlic was purchased and collected from Al-local Nasiriya's market in Iraq. The plants were thoroughly cleaned and washed in sterile distilled water before being dried at room temperature and milled into a fine powder using an electric blender. One hundred grams (100g) of each powdered plant material was extracted separately in a Soxhlet apparatus for 8 hours with 500 mL of 70% ethanol. Aqueous extract was also extracted using this procedure. The Whatman No. 1 filter paper was used to filter all of the extracts. The filtrates were then evaporated at 40°C using a rotary evaporator under reduced pressure, and the resultant dry extracts were stored at 4°C in sterilized flasks until used to prepare 150 mg/mL from each extract (Mukhtar et al., 2017).

Evaluation the antibacterial activity of plants

The modified agar well-diffusion method was used in this study to evaluate the antibacterial activities of the extracts. 0.1mL of standardized *Serratia marcescens* inoculums (1.5×10^8 CFU/mL) was aseptically spread onto the surface of sterile Mueller Hinton agar and allowed to dry for 30 minutes. Using a sterile stainless-steel borer, create 8 mm diameter wells in agar plates containing the bacteria and fill with 50 µl of each extract. The prepared plates were left at room temperature for 30 minutes to allow the extracts to diffuse into the agar before being incubated for 24 hours at 37°C. The antibacterial activity was determined by measuring the diameter of the inhibition zone produced by the extracts against the tested bacteria (Unnisa et al., 2012).

Antimicrobial Sensitivity tests

The susceptibility of the isolates under consideration was investigated using (Stocks, and Ridgway, 1987) by using Muller - Hinton agar, and the results were interpreted in accordance with (CLSI, 2009) and the following antibiotics discs were used: Cephalothin, Cefotaxime, Cefaclor, Ceftazidime and Amoxicillin-clavulanate.

Results

Out of 100 swabs and samples collected from blood specimens of infant patients, bacterial growth was identified in 22 cultures (22%). The results showed that after treating 22 *Serratia* isolates with antibiotics, 22 isolates were identified as *Serratia* producing ESBLs and resistant 100% to antibiotics after incubating the plates cultured with *Serratia* bacteria for a whole isolate as in figure (1)

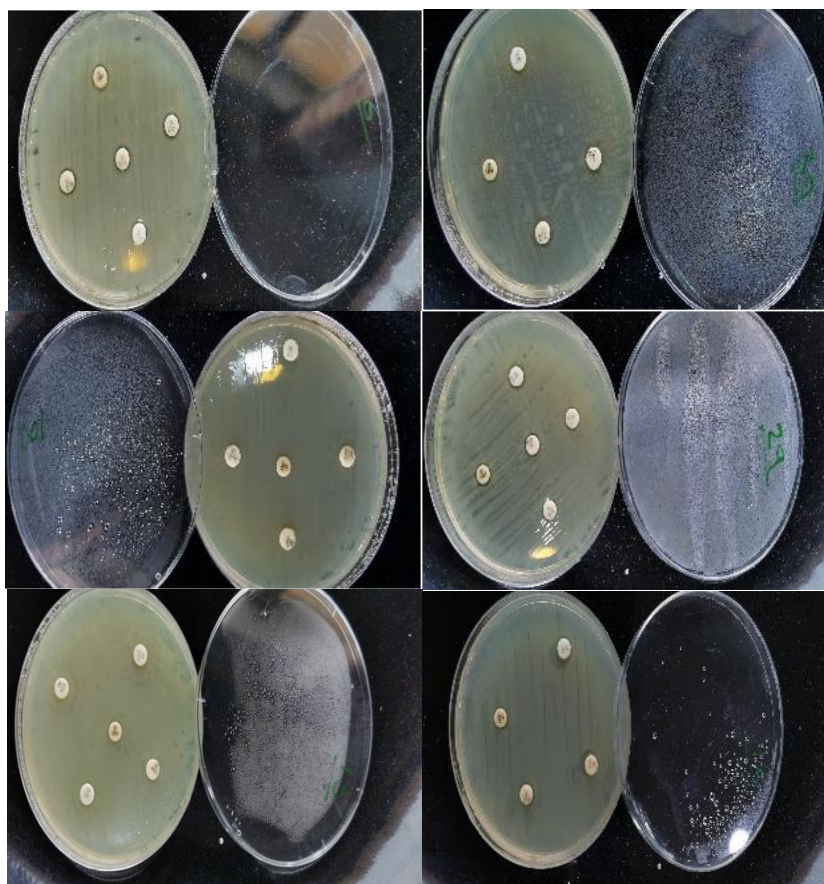


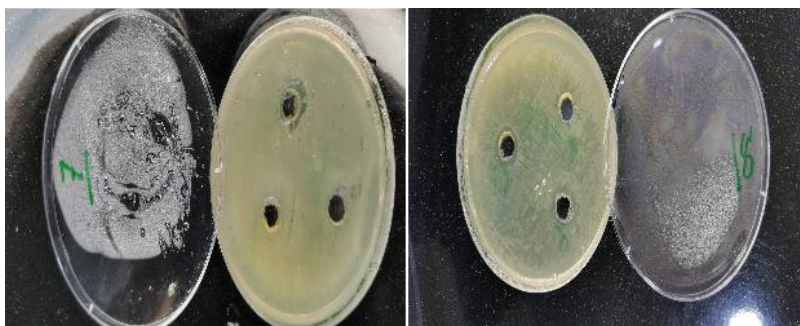
Figure 1. Pictures showing the resistance of *Serratia* bacteria to the antibiotics and its production of ESBLs

We diagnosed one species of *S. marcescens* among the isolates in our study and the prevalence of positive ESBL among these strains was positive, and the rate of ESBL production in *Serratia* strains was consistent with that in a previous study (molecular evaluations of the extended-spectrum β -lactamase-producing strains of *Serratia* isolated from samples The blood of patients in Namazi Hospital, Shiraz, southern Iran) has a high degree of resistance (Lii 2004), and the results also indicate that due to the emergence of ESBL strains, third and fourth generation cephalosporins cannot be relied upon in the treatment of diseases caused by *Serratia* strains. The results showed that there were inhibition rates after treating 22 isolates of *Serratia* with garlic extract (hexane, alcohol, hexane and alcohol mixtures), where 65% of the samples showed inhibition in different

proportions, and 35% showed resistance and inhibition percentages appeared only with alcohol and as for hexanb and hexane and alcohol mixtures. No results appeared isolates as in table (2) and figure. (2)

Table 2
Inhibiting levels of garlic extracts

Sample	Isolation number	Inhibition rates of alcohol	Inhibition rates of Hexan	Inhibition rates of Hexan & alcohol
1	1	%13	%0	%0
2	2	%0	%0	%0
3	6	%10	%0	%0
4	7	%13	%0	%0
5	8	%18	%0	%0
6	9	%0	%0	%0
7	10	%0	%0	%0
8	13	%14	%0	%0
9	14	%0	%0	%0
10	15	%14	%0	%0
11	17	%0	%0	%0
12	30	%13	%0	%0
13	19	%14	%0	%0
14	22	%0	%0	%0
15	23	%13	%0	%0
16	24	%11	%0	%0
17	25	%10	%0	%0
18	26	%14	%0	%0
19	28	%0	%0	%0
20	29	%10	%0	%0
21	20	%12	%0	%0
22	21	%0	%0	%0



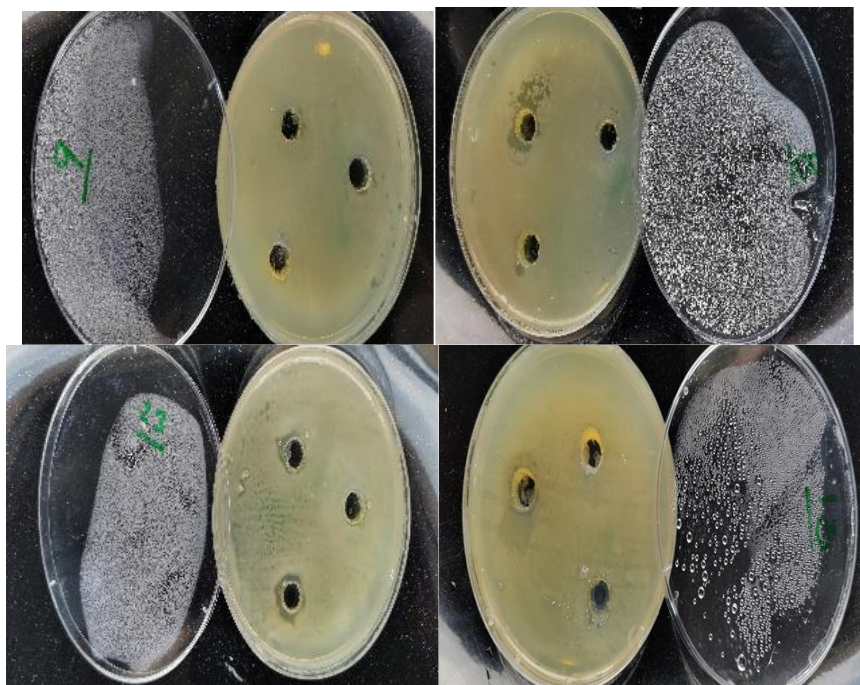


Figure 2. Agricultural dishes after treatment with garlic extracts

Discussions

Serratia marcescens is a late lactose fermenter on MacConkey agar, and it may produce red prodigiosin if the plate is incubated at 25°C (Winn, 2006). One of the secondary metabolites produced by *S. marcescens* isolates is prodigiosin (Stella et al., 2018). *S. marcescens* is a common species in clinical practice, causing urinary tract infection, meningitis, pneumonia, infective endocarditis, catheter-associated bloodstream infection, wound infection, and other infections (Marin et al., 2017). *Serratia marcescens* is well-known for causing nosocomial infections, particularly bloodstream infections, in the NICU (Cristina et al., 2019). A study conducted in Algeria by Batah et al., (2015) appeared to be compatible with the current study's findings in that they reported that (100%) of their *Serratia marcescens* isolates were resistant to the antibiotic amoxicillin/clavulanic acid.

Another study, on the other hand, did not appear to be consistent with our findings. According to Simsek (2019), an antibiotic susceptibility test was performed on *S. marcescens* isolated from various sources, and the antibiotics with the lowest resistance rates were determined to be cefotaxime and gentamicin (0.6 percent). Multidrug-resistant (MDR) and extensively drug-resistant (XDR) *S. marcescens* infections are on the rise in a number of hospital wards around the world, posing a serious public health concern (Rodríguez et al., 2017). Although *Serratia marcescens* has intrinsic resistance to ampicillin and colistin, clinical isolates with pan drug resistance are uncommon (Moradigaravand et al., 2016). *Serratia marcescens* is intrinsically resistant to ampicillin and colistin, but clinical isolates of *S. marcescens* with pan drug resistance are uncommon. It is worth noting that the variation in the levels of resistance of bacterial isolates to

antimicrobials is due to a variety of factors such as geographical differences, the size of studied samples, the site of infection, and the source of specimens (Watts et al., 2018). Maragakis et al., (2008), reported investigation and containment of an outbreak of multidrug-resistant (MDR) *S. marcescens* infection at a NICU, which was consistent with the findings of the current study.

Between 2001 and 2011, there were reports of the rise of multidrug-resistant *S. marcescens* in the United Kingdom and Ireland, which is a major issue for future treatment of this bacterial illness (Moradigaravand et al., 2016). Allicin, the active ingredient of garlic, acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target (Eja, et al., 2007). The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids (Griffiths, et al., 2002). Organ sulfur compounds and phenolic compounds have been reported to be involved in the garlic antimicrobial activity (Jombo, et al., 2011; Aboaba, and Efuwape, 2001). The garlic cutting and crushed in during the preparation of fresh juice increases the contact surfaces and then edit the active compounds (Dankert, 1979). For this reason, we note that fresh juice gave effective against microbial more of the aqueous extract and antibiotics used.

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