Antimicrobial Resistance Properties of Staphylococcus Aureus Isolates From Powdered Packaged Medicinal Plants and Bottle Herbal Distillates

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Research

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Abstract

Background

Human involvement in the production and processing of medicinal plants and herbal distillates caused a potential risk of microbial contamination, particularly with *Staphylococcus aureus*. The present research was performed to assess the prevalence and phenotypic and genotypic properties of antibiotic resistance of *S. aureus* bacteria isolated from diverse kinds of powdered packaged medicinal plant and bottle herbal distillate samples.

Methods

Three-hundred different powdered packaged medicinal plant and bottle herbal distillate samples produced in traditional conditions were collected and examined by the culture method. Phenotypic and genotypic patterns of antibiotic resistance of *S. aureus* isolates were examined using disk diffusion and PCR techniques.

Results

Thirty out of three-hundred (10%) powdered packaged medicinal plant and bottle herbal distillate samples were contaminated with *S. aureus*. The prevalence of *S. aureus* amongst the powdered packaged medicinal plant and bottle herbal distillate samples were 8.33% and 11.11%, respectively. *A. citrodora* (10%) and *R. damascene* (10%) powdered packed medicinal plants and *A. maurorum* (16.66%) bottle herbal distillate had the highest contamination rate with *S. aureus*. *S. aureus* isolates harbored the highest prevalence of resistance toward penicillin (93.33%), tetracycline (90%), gentamicin (86.66%), erythromycin (70%), trimethoprim-sulfamethoxazole (63.33%) and ciprofloxacin (53.33%). Totally, 13.33% of the *S. aureus* isolates harbored resistance toward more than 7 antibiotic agents. *blaZ* (63.33%), *tetK* (60%), *ermA* (46.66%), *msrA* (43.33%), *aacA-D* (43.33%), and *mecA* (43.33%) were the most frequent antibiotic resistance genes.

Conclusions

Powdered packaged medicinal plant and bottle herbal distillate samples may be sources of multidrug resistant-*S. aureus,* which poses a hygienic threat concerning the consumption of these therapeutic options in Iran. Nevertheless, further research is compulsory to understand other epidemiological features of *S. aureus* in powdered packaged medicinal plant and bottle herbal distillate samples.

Background

Medicinal plants and herbal distillates are rich sources of therapeutic agents with high beneficial effects on human health. Recently, the acceptance of medicinal plants and herbal distillates is improved to 20% of the world population [1]. Moreover, dome kinds of medicinal plants and herbal distillates are mainly used as flavors and additives in the food industries [2]. Furthermore, about 70 to 80 percent of developing countries use herbal and traditional medicine for their primary health care [3]. Diverse kinds of medicinal plants including *Zatariamultiflora* (*Z.multiflora*), *Saturejabachtiarica* (*S. bachtiarica*), *Aloysiacitrodora* (*A.citrodora*), *Rosadamascene* (*R.damascene*), *Lavandulaangustifolia* (*L.angustifolia*), *Alhagi maurorum* (*A. maurorum*), *Cichorium intybus* (*C. intybus*), *Melissaofficinalis* (*M.officinalis*), *Menthapiperita* (*M.piperita*), and *Fumariaofficinalis* (*F.officinalis*) are extensively used as antimicrobial, antioxidant, anticancer, anti-neoplasia, food additive, anti-inflammation, wound healing, antiseptic, anti-diabetic, diuretic, expectorant, stimulating the central nervous system, digestive, anti-mutagenic, sedative, analgesic, etc. agents among people al-around the world [1-9]. They also have an export aspect. Thus, it is essential to ensure the quality and safety of these products.

Human involvement in the packaging, powdering, and further procedures of medicinal plants and preparation of bottle herbal distillates caused their unintentional microbial contamination, particularly with those originated from the upper respiratory tract and skin [10]. Previous surveys determined the *Staphylococcusaureus* (*S. aureus*) in diverse kinds of medicinal plants and herbal distillates [11]. It is a Gram-positive, catalase-positive, and cocci-shaped bacterium with high attendance on the skin and upper respiratory tract [11]. The bacterium is responsible for plain nosocomial and community-acquired infections, foodborne diseases, and food poisoning [12-15]. Food-related diseases and disorders caused by *S. aureus* are mainly known by abdominal cramps, nausea, vomiting, weakness, and diarrhea, and also toxic shock syndrome (TSS) [11, 12]. Foodborne diseases caused by the *S. aureus* bacteria are faced with some difficulties in treatments [13]. Some *S. aureus* isolates mainly resist against diverse antibiotic agents, particularly penicillins, cephems, glycopeptides, aminoglycosides, macrolides, and tetracyclines fluoroquinolones, nitrofurantoins, lincosamides, folate pathway antagonists, phenicols, ansamycins, and even streptogramins [14, 15]. Epidemiological surveys determined that antibiotic resistance encoding genes are among the main reasons for antibiotic resistance in *S. aureus* bacteria [16, 17]. Considerable prevalence of the genes that encode resistance toward cephalosporins (*blaCTX-M*), penicillins (*mecA* and *blaZ*), glycopeptides (*vanA* and *vanB*), aminoglycosides (*aacA-D*), macrolides (*ermA*, *ermB*, *msrA*, and *msrB*), tetracyclines (*tetK* and *tetM*), folate pathway antagonists (*dfrA1*), ansamycins (*rpoB*), lincosamides (*linA*), fluoroquinolones (*gyrA* and *grlA*), phenicols (*fexA*) and streptogramins (*vatA* and *vatB*) have been reported in the *S. aureus* bacteria recovered from clinical and food samples [16, 17]. Antibiotic resistant-*S. aureus* strains caused more severe clinical diseases with higher morbidity and mortality rates for a longer period, which accommodate higher economic loads of control, prevention, and treatments [18].

According to people's general perception of medicinal plants' healing effects and herbal distillates, there is a big paradox regarding their microbial contamination. Therefore, it is substantial to assess the microbial quality of medicinal plants and herbal distillates. Thus, the present survey was conducted to assess the prevalence rate and phenotypic and genotypic assessment of *S. aureus* bacteria's antibiotic resistance isolated from different powdered packaged medicinal plant and bottle herbal distillate samples produced in traditional producing units in Iran.

Materials And Methods

Sampling

From May 2019 to January 2020, a total of 300 diverse kinds of powdered packaged medicinal plants including *Z. multiflora* (n= 30), *S.bachtiarica* (n= 30), *A.citrodora* (n= 30) and *R.damascene* (n= 30) and bottle herbal distillates including *L.angustifolia* (n= 30), *A. maurorum* (n= 30), *C. intybus* (n= 30), *M. officinalis* (n= 30), *M.piperita* (n= 30) and *F.officinalis* (n= 30) were randomly collected from shopping centers, Tehran, Iran. A total of 50 g samples were collected from each powdered packaged medicinal plant and bottle herbal distillate sample using a sterile laboratory tube. All bootle herbal distillates were produced conventionally in small traditional producing units. Additionally, all collected powdered packaged medicinal plants were dried, powdered, and packed conventionally in traditional production units. Specifications about samples were recorded according to their labels. All samples were directly transferred to the laboratory at 4 °C.

Isolation and identification of S. aureus bacteria

Twenty-five grams of each collected powdered packaged medicinal plant and bottle herbal distillate samples were blended with 225 mL of buffered peptone water (Merck, Germany). At that time, solutions were homogenized using Stomacher (Interscience, Saint-Nom, France). At that point, five milliliters of the achieved solution was transferred into 50 mL Trypticase Soy Broth (TSB, Merck, Germany) supplemented with 10% NaCl and 1% sodium pyruvate and incubated for 18 h at 35 °C. At that moment, a loopful of the culture was transferred into Baird-Parker agar supplemented with egg yolk tellurite emulsion (Merck, Germany) and incubated at 37 °C for about 24 h. Black shiny colonies enclosed with significant zones identified using biochemical tests including Gram staining, oxidase test, catalase activity, resistance to bacitracin (0.04 U), coagulated test (rabbit plasma), urease activity, glucose O/F test, voges-proskaver (Merck, Germany) test, nitrate reduction, phosphatase, deoxyribonuclease (DNase, Merck, Germany) test, mannitol fermentation, hemolysis activity on blood agar (Merck, Germany) and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests [19].

Phenotypic analysis of antibiotic resistance of S. aureus isolates

The phenotypic pattern of antibiotic resistance of *S. aureus* bacteria isolated from powdered packaged medicinal plant and bottle herbal distillate samples was assessed using the disk diffusion method using the Mueller–Hinton agar (Merck, Germany) medium. Ideologies of Clinical and Laboratory Standards Institute (CLSI) were used for this goal [20]. Diverse kinds of antibiotic groups including penicillins (oxacillin (1 µg/disk) and penicillin (10 units/disk)), cephems (cefoxitin (30 µg/disk) and ceftaroline (30 µg/disk)), glycopeptides (vancomycin (5 µg/disk)), aminoglycosides (gentamicin (15 µg/disk)), macrolides (azithromycin (15 µg/disk) and erythromycin (15 µg/disk)), tetracyclines (tetracycline (30 µg/disk) and doxycycline (30 µg/disk)), fluoroquinolones (ciprofloxacin (5 µg/disk) and levofloxacin (5 µg/disk)), nitrofurantoins (nitrofurantoin (300 µg/disk)), lincosamides (clindamycin (2 µg/disk)), folate pathway antagonists (trimethoprim-sulfamethoxazole (1.25/23.75 µg/disk)), phenicols (chloramphenicol (30 µg/disk)), ansamycins (rifampin (5 µg/disk)) and streptogramins (quinupristin-dalfopristin (15 µg/disk)) were applied for this goal (Oxoid, UK). The method was performed using the way designated previously [20]. *S. aureus* ATCC 25923 was used as control.

DNA extraction and quality examination

S. aureus isolates were sub-cultured on TSB media (Merck, Germany) and incubated for 48 h at 37 ^{o}C. Genomic DNA was extracted from MRSA colonies using the DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany). Guidelines of the producing company were performed for this purpose. Purity (A260/A280) of extracted DNA was examined by the NanoDrop device (NanoDrop, Thermo Scientific, Waltham, MA, USA). The quality of extracted DNA was examined using electrophoresis on 2% agarose gel.

Genotypic analysis of antibiotic resistance of S. aureus isolates

The genotypic pattern of antibiotic resistance of *S. aureus* bacteria isolated from powdered packaged medicinal plant and bottle herbal distillate samples was assessed by PCR technique [21-28]. Distribution of the genes that encode resistance against cephems (*blaCTX-M*), penicillins (*blaZ* and *mecA*), glycopeptides (*vanA* and *vanB*), aminoglycosides (*aacA-D*), macrolides (*ermA*, *ermB*, *msrA* and *msrB*), tetracyclines (*tetK* and *tetM*), folate pathway antagonists (*dfrA1*), ansamycins (*rpoB*), lincosamides (*linA*), fluoroquinolones (*gyrA* and *grlA*), phenicols (*fexA*), and streptogramins (*vatA* and *vatB*) antibiotics were studied. Table 1 discloses the PCR circumstances used for the detection of antibiotic resistance genes amongst the MRSA strains. A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was applied for this goal. Amplified products were analyzed by electrophoresis (120 V/208 mA) in 2.5% agarose gel. The gel was stained with 0.1% ethidium bromide (0.4 µg/ml, (Thermo Fisher Scientific, St. Leon-Rot, Germany). The UVI doc gel documentation systems (Grade GB004, Jencons PLC, London, UK) was used to assess the results of the PCR.

Statistical analysis

Statistical analysis was done using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between the phenotypic and genotypic properties of antibiotic resistance of the *S. aureus* bacteria isolated from powdered packaged medicinal plant and bottle herbal distillate samples. *P*-value <0.05 was considered as significant statistical level.

Results

Contamination rate of examined samples with S. aureus

The present survey was conducted to assess the prevalence and phenotypic and genotypic examination of *S. aureus* bacteria's antibiotic resistance isolated from diverse kinds of powdered packaged medicinal plant and bottle herbal distillate samples.

Table 2 shows the contamination rate of diverse powdered packaged medicinal plant and bottle herbal distillate samples with *S. aureus*. Thirty out of three-hundred (10%) medicinal plant and herbal distillate samples were contaminated with *S. aureus*. The contamination rates of powdered packaged medicinal plant and bottle herbal distillate samples were 8.33% and 11.11%, respectively. *A.citrodora* (10%) and *R.damascene* (10%) had the highest contamination rate with *S. aureus* amongst all examined powdered packaged medicinal plants, while *S.bachtiarica* (6.66%) and *Z.multiflora* (6.66%) had the lowest. *A. maurorum* (16.66%) had the highest contamination rate with *S. aureus* amongst all examined bottle herbal distillates, while *M.officinalis* (6.66%) and *M.piperita* (6.66%) had the lowest. Statistically significant difference was obtained for the contamination rate of *S. aureus* between powdered packaged medicinal plants and bottle herbal distillates (P <0.05). Additionally, statistically significant differences were obtained between examined samples and the contamination rate with *S. aureus* (P <0.05).

Phenotypic properties of antibiotic resistance

Table 3 shows the phenotypic pattern of antibiotic resistance of *S. aureus* bacteria isolated from diverse kinds of powdered packaged medicinal plant and bottle herbal distillate samples. *S. aureus* bacteria exhibited the highest prevalence of resistance toward penicillin (93.33%), tetracycline (90%), gentamicin (86.66%), erythromycin (70%), trimethoprim-sulfamethoxazole (63.33%), ciprofloxacin (53.33%), oxacillin (50%), and cefoxitin (50%) antibiotic agents. Reversely, *S. aureus* bacteria harbored the lowest prevalence of resistance toward chloramphenicol (20%), nitrofurantoin (36.66%), quinupristin-dalfopristin (40%), azithromycin (43.33%), doxycycline (43.33%), and rifampin (43.33%) antibiotic agents. *S. aureus* bacteria isolated from bottle herbal distillates harbored a higher and more diverse prevalence of resistance toward examined antibiotic agents (P <0.05). Additionally, a statistically significant difference was obtained between types of examined samples and prevalence of antibiotic resistance (P <0.05).

Prevalence of multidrug resistant-S. aureus

Figure 1 shows the prevalence of multidrug resistant-*S. aureus* bacteria amongst all examined samples. All of the *S. aureus* bacteria isolated from powdered packaged medicinal plant and bottle herbal distillate samples harbored resistance to at least one of the examined antibiotic agents. The prevalence of resistance toward at least five antibiotic agents was 46.66%. Findings revealed that 13.33% of the *S. aureus* isolates harbored resistance toward more than seven antibiotic agents.

Genotypic properties of antibiotic resistance

Table 4 shows the genotypic pattern of antibiotic resistance of *S. aureus* bacteria isolated from diverse kinds of powdered packaged medicinal plant and bottle herbal distillate samples. The most prevalent antibiotic resistance genes amongst the *S. aureus* bacteria isolated from examined powdered packaged medicinal plant and bottle herbal distillate samples were *blaZ* (63.33%), *tetK* (60%), *ermA* (46.66%), *msrA* (43.33%), *aacA-D* (43.33%), and *mecA* (43.33%). Distribution of *msrB* (6.66%), *ermB* (10%), *vanB* (13.33%), *fexA* (13.33%), *rpoB* (20%), and *vatB* (20%) were lower than other examined antibiotic resistance genes. *S. aureus* bacteria isolated from bottle herbal distillates harbored the higher and more diverse distribution of antibiotic resistance genes (P <0.05). Additionally, a statistically significant difference was obtained between types of examined samples and distribution of antibiotic resistance genes (P <0.05). Statistical significant differences were obtained between the distribution of *mecA* and *blaZ* (P <0.05), *vanA* and *vanB* (P <0.05), *vatA* and *vatB* (P <0.05), *ermA* and *ermB* (P <0.05), *msrA* and *msrB* (P <0.05) and *tetK* and *tetM* (P <0.05) antibiotic resistance genes. There was no significant difference between the distribution of fluoroquinolones resistance genes (P >0.05).

Discussion

Extensive diversity of microbial contaminants might accompany medicinal plants. Unavoidably, the microbial background relies on numerous ecological factors and employs an imperative influence on herbal products' safety and quality. The microbial contaminants of herbal products are simply transferred through air, soil, animal- and human-based fertilizers, and finally infected staff and workers producing units [10]. Otherwise, a host of agricultural, environmental, industrial, and urban factors, together with less than good harvesting, storage, and processing procedures, are additional reasons for contamination in herbal products [10]. In these cases, medicinal plants and herbal products with confirmed therapeutic effects not only do not improve the patient's condition, but also lead to diverse kinds of foodborne diseases and disorders. Thus, assess the microbial quality of herbal products is essential as an imperative public health matter.

The present survey was conducted to assess the prevalence and antibiotic resistance properties of the *S. aureus* bacteria isolated from diverse kinds of powdered packaged medicinal plant and bottle herbal distillate samples produced in traditional circumstances in Iran. Findings of the current investigation disclosed that the contamination rate of examined raw medicinal plants and herbal distillates with *S. aureus* was 10%. Some of the examined samples, such as *Z.multiflora* (6.66%) and *S.bachtiarica* (6.66%) powdered packaged medicinal plants and *M.officinalis* (6.66%), and *M.piperita* (6.66%) bottle herbal distillates had the lower contamination rate with *S. aureus*. One of the probable reasons for this finding is the high antimicrobial effects of *Z.multiflora*, *S.bachtiarica*, *M.officinalis*, *M.piperita,* and *C. intybus* against diverse kinds of bacteria [29-32]. Thus, *S. aureus*' growth and survival have been decreased and even stopped in these medicinal plants and their derived products.

S. aureus is most expected to originate from herbal products' contact with food handlers throughout harvesting, processing, and storage, and its absence reflects the acceptable hygiene practices. Our findings also revealed that herbal distillates had a higher contamination rate with *S. aureus* than medicinal plants. The probable reason for this finding is maybe the extinction of *S. aureus* bacteria during medicinal plants' drying process. Additionally, the processing of herbal distillates requires more human involvement and manipulation. Thus, the transmission of *S. aureus* bacteria from the infected staff and workers producing units to the herbal distillates may be another reason for the high prevalence of *S. aureus* in these samples. Despite the high importance of the topic, many limited surveys have been conducted in this field. A survey concocted by Sousa Lima et al. (2020) [33] disclosed that the prevalence of *S. aureus* bacteria amongst the homemade and commercial herbal medicine samples (*Lippia alba*, *Peumus boldus Molina*, *Cymbopogon citratus*, *Carapa guianensis*,

Copaifera langsdorffii, *Stryphnodendron adstringens*, *Costus spicatus*, and *Arrabidaea chica*) was 88.50% and 23.50%, respectively. Kaume et al. (2012) [34] described that the prevalence of *S. aureus* amongst the medicinal plants marketed to patients suffered from the HIV-infection in Kenya was 71.40%, which was entirely higher than our findings. Esimone et al. (2007) [35] also reported that the prevalence of *S. aureus* amongst the medicinal plants sold in Nigeria was 8.70%. Reversely, No *S. aureus* strains bacteria were recovered from the medicinal plants in the study conducted in South-Africa [36]. Ideh et al. (2019) [37] described that the Staphylococcal microbial load of some kinds of medicinal plants in Nigeria had ranged between 1.50×10^5 to 6.75×10^6 Colony Forming Unit (CFU)/g, which was entirely higher than the limit microbial load introduced by the World Health Organization (WHO) (10^4 CFU/g) [38]. Similarly, a high contamination rate of herbal products with *S. aureus* and other Staphylococcal species has been reported previously from Bangladesh [39], Korea [40], Nigeria [41, 42], Germany [43], Sudan [44], Saudi Arabia [45], Benin [46], and Ethiopia [47]. Likewise, high microbial contamination of some kinds of medicinal plants other than *S. aureus* has been reported in diverse researches conducted on Iran [48, 49], Bangladesh [50], Tanzania [51], Kenya [52, 53], Malaysia [54], Iraq [55], Poland [56, 57], Italy [58], Saudi Arabia [59], Thailand [60], South Africa [61], Pakistan [62], and United States [63]. One possible reason for the presence of diverse bacteria, particularly *S. aureus*, in powdered packaged medicinal plant and bottle herbal distillate samples in their proper intrinsic factors such as pH. The pH levels of examined powdered packaged medicinal plant and bottle herbal distillate samples ranged from 3.5 to 9.5, facilitating luxuriant growth and survival of most bacterial species [64]. The contamination rate of herbal product samples with *S. aureus* vary between diverse researches. The difference in data advises that time, season, place of sampling, method of sampling, types of samples, and even laboratory techniques applied in research may affect surveys' outcomes. Moreover, difference hygienic levels of producing units of herbal products may affect the prevalence of *S. aureus* in diverse investigations. Compared to the results of other scientists, the comparatively low rate of *S. aureus* isolation was reported in our survey. Relatively low contamination rate may be due to natural antimicrobials and possibly to a commonly good hygiene situation, however high contamination rate may specify less favourable hygienic circumstances.

The second part of the present survey was performed on the antibiotic resistance properties of *S. aureus* isolates. Findings revealed that *S. aureus* bacteria displayed the highest prevalence of resistance toward penicillin, tetracycline, gentamicin, erythromycin, trimethoprim-sulfamethoxazole, ciprofloxacin, and cefoxitin antibiotic agents, which was accompanied by high prevalence of *blaZ* and *mecA*, *tetK*, *aacA-D*, *msrA* and *ermA*, gyrA, *dfrA1,* and *blaCTX-M* antibiotic resistance encoding genes, respectively. Thus, the phenotypic presence of antibiotic resistance was confirmed by the genotypic presence of antibiotic resistance encoding genes. Furthermore, the presence of multidrug resistant-*S. aureus* was found in some isolates. Irregular and unauthorizing antibiotic agents' prescription is the probable reason for the high prevalence of resistance and high distribution of antibiotic resistance genes. Findings disclosed that some *S. aureus* bacteria exhibited a higher prevalence of resistance toward antibiotic agents used to treat human clinical infections, which can indirectly signify that they may transmit from infected staff and workers of producing units of medicinal plants and herbal distillates. Reversely, some others exhibited a higher prevalence of resistance toward antibiotics used mostly for treatment of animal infections, which can indirectly demonstrate that they may transmit from animal species, particularly in using animal-based fertilizers and polluted water for growth and irrigation of medicinal plants. The presence of resistance toward chloramphenicol (20%), which was assisted with attendance of *fexA* antibiotic encoding gene (13.33%) may reflect using of poultry-based fertilizers for the growth of medicinal plants since chloramphenicol is a common antibiotic choice in Iranian poultry farms [65, 66]. According to the literature, the present survey is the first report of the phenotypic and genotypic assessments of antibiotic resistance amongst the *S. aureus* bacteria isolated from powdered packaged medicinal plants and bottle herbal distillate samples globally. Braide et al. (2013) [67] stated that the *S. aureus* bacteria isolated from herbal remedies were susceptible to ofloxacin, chloramphenicol, gentamicin, pefloxacin, ciprofloxacin, and erythromycin antibiotic agents. Ngemenya et al. (2019) [68] described that the *S. aureus* strains isolated from herbal remedies in Cameroon were resistant against five classes of examined antibiotic agents (amikacin, cefotaxime, cefuroxime, imipenem, trimethoprim, and ceftriaxone). Similarly, Yesuf et al. (2016) [47] reported that the *S. aureus* strains isolated from medicinal herbal products in Ethiopia harbored the high prevalence of resistance toward ampicillin (80%), penicillin (60%), amoxicillin (40%), amoxicillin-clavulanic acid (40%), chloramphenicol (40%), and cloxacillin, (30%). Similar pattern of resistance of *S. aureus* bacteria has been described toward penicillins [66, 69-73], tetracyclines [66, 69-71], aminoglycosides [66, 69-73], macrolides [66, 69-73], cephems [66, 69-73], fluoroquinolones [66, 69-73], and folate pathway antagonists [66, 69-73] antibiotic groups. A similar resistance pattern of the *S. aureus* bacteria was reported previously [74, 75]. Differences in the opinion of medical and veterinary practitioners in an antibiotic prescription, observation of ethics and rules in the use of antibiotics, availability or lack of antibiotics, and their prices are probable reasons for differences found in the prevalence of resistance of *S. aureus* strains in numerous investigations. Diverse researches have been conducted to appraise the antibiotic resistance properties of *S. aureus*. Most of them reported the confirmation of phenotypic pattern of antibiotic resistance by the presence of diverse antibiotic encoding genes [16, 66, 76, 77] High distribution of *blaZ*, *mecA*, *tetK*, *aacA-D*, *msrA*, *ermA*, *gyrA*, *dfrA1,* and *blaCTX-M* antibiotic resistance encoding genes in the *S. aureus* bacteria isolated from diverse kinds of food samples and also human clinical infections have been reported from Iran [66, 76, 78], India [79], Georgia [72], Nigeria [80], Germany [81], Egypt [82], and Switzerland [83]. Comparable to our research, higher prevalence of *msrA* than *msrB* [66, 76, 78, 84], *ermA* than *ermB* [66, 76, 78, 84, 85], *tetK* than *tetM* [66, 76, 78, 84] *vatA* than *vatB* [66, 76, 78, 84], *vanA* than *vanB* [66, 76, 78, 84, 85] and *gyrA* than *grlA* [66, 76, 84] antibiotic resistance encoding genes has been reported in recent years. Our findings were also disclosed a higher prevalence of phenotypic profile of resistance than genotypic profile. For instance, all of the penicillin-resistant *S. aureus* bacteria didn't harbored *blaZ* and *mecA* antibiotic resistance genes. This matter was also existed for other antibiotic agents and resistance genes. This finding is maybe owing to the fact that presence of antibiotic resistance genes is one of the known procedures for occurrence of antibiotic resistance in bacteria. In the other hand, several mechanisms have been identified to induce antibiotic resistance in bacteria including reduced permeability of bacteria to antibiotics, efflux antibiotic's active pumps to out of the bacterial cell, change in antibiotic target site, inactivation of antibiotics through hydrolysis or changes in their structure, occurrence of genetic mutations and access of bacteria to the secondary metabolic pathways that compensate the antibiotic-inhibited reactions.

Findings also revealed the high prevalence of multidrug resistant-*S. aureus* strains amongst medicinal plants and herbal distillates. Similarly, a high prevalence of multidrug-resistant bacteria has been reported in herbal product samples in Kenya [86], Cameroon [68], and Ethiopia [47]. Additionally, the high distribution of multidrug resistant-*S. aureus* strains have been described in clinical specimens and foodstuff samples collected from China [87], United States [88], and Kuwait [89]. Altogether, the high prevalence of antibiotic resistance in *S. aureus,* which was accompanied by the high distribution of antibiotic resistance genes and the presence of multidrug resistance, disclosed a pressing public health issue regarding the consumption of powdered packaged medicinal plants and bottle herbal distillate samples.

Conclusions

Put together, an existing survey is the first report of prevalence and phenotypic evaluation of antibiotic resistance of *S. aureus* bacteria isolated from *Z. multiflora*, *S.bachtiarica*, *A.citrodora* and *R.damascene* powdered packaged medicinal plants and *L.angustifolia*, *A. maurorum*, *C. intybus*, *M. officinalis*, *M.piperita* and *F.officinalis* bottle herbal distillates, globally. Additionally, it was the first report of detection of antibiotic resistance genes amongst the *S. aureus* strains isolated from diverse kinds of powdered packaged medicinal plants and bottle herbal distillate samples globally. Findings disclose that powdered packaged medicinal plants and particularly bottle herbal distillates are potential sources of multidrug resistant-*S. aureus*. Bottle herbal distillates harbored a higher prevalence of *S. aureus* isolates and also higher antibiotic resistance. High prevalence of resistance of *S. aureus* bacteria toward penicillin, tetracycline, gentamicin, erythromycin, trimethoprim-sulfamethoxazole, ciprofloxacin, and cefoxitin antibiotic agents was observed, which was also accompanied by the high prevalence of *blaZ* and *mecA*, *tetK*, *aacA-D*, *msrA* ans *ermA*, gyrA, *dfrA1* and *blaCTX-M* antibiotic resistance encoding genes, respectively. The prevalence of resistance toward human-based antibiotics and animal-based antibiotics can indirectly show *S. aureus* isolates' origin. It seems that penicillin, tetracycline, gentamicin, erythromycin, trimethoprim-sulfamethoxazole, ciprofloxacin, and cefoxitin are not effective therapeutic agents in the cases of *S. aureus* foodborne diseases at this time in Iran. Types of examined samples had high effects on the prevalence and antibiotic resistance properties of *S. aureus* strains. Traditional producing and processing centers of powdered packaged medicinal plants and bottle herbal distillates can be severely contaminated with foodborne pathogens, particularly *S. aureus*; the maintenance of their hygiene, regular microbiological monitoring of these samples, implementation of good manufacturing practices and a food safety system such as the Hazzard Analysis Critical Control Points (HACCP) system are essential to minimize the risk to the consumer. Furthermore, using high quality raw materials in production of powdered packaged medicinal plants and bottle herbal distillate samples, prevention from cross-contamination and antibiotic prescription based on disk diffusion outcomes can diminish the risk of transmission of multidrug resistant-*S. aureus* bacteria from powdered packaged medicinal plants and bottle herbal distillate samples to the human population. On the basis of these observations, we recommend that attention should be paid by governments and individuals to prevent the further spread of multidrug resistant-*S. aureus*. However, supplementary surveys are essential to determine more epidemiological features of the multidrug resistant-*S. aureus* bacteria in powdered packaged medicinal plants and bottle herbal distillate samples.

Abbreviations

S. aureus: *Staphylococcus aureus*; PCR: Polymerase Chain Reaction; SPSS: Statistical Package for the Social Sciences

Declarations

Ethics approval and consent to participate

The present investigation was confirmed by the ethical supervision of Scientific Research of the Alborz, University of Medical Sciences, Karaj, Iran (No 3607614). Sampling licenses were taken by Dr. Bahareh Tavakoli-Far and Dr. Zohreh Mashak (Ref Number 3607614).

Consent for publication

There was no consent for publication.

Availability of data and material

All data generated or analyzed throughout this research are included in this published article.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

BT designed the study and carried out the culture-based identification and PCR genetic alignment. ZM supported the study and carried out the sample collection, disk diffusion and statistical analysis. BT M carried out the writing and drafting of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. PCR conditions to amplify antibiotic resistance genes amongst the *S. aureus* bacteria isolated from powdered packaged medicinal plants and bottle herbal distillate samples.

Target gene	Encoding antibiotic	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
aacA-D	Aminoglycosides	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227	1 cycle: 94 ^{0C} ——– 5 min. 25 cycles: 94 ^{0C} ——– 60 s 55 ^{0C} ——– 70 s 72 ^{0C} ——– 60 s 1 cycle: 72 ^{0C} ——– 10 min	5 µL PCR buffer 10X 1.5 mM $MgCl_2$ 200 µM dNTP (Thermo Fisher Scientific, St. Leon-Rot, Germany) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Thermo Fisher Scientific, St. Leon-Rot, Germany) 2.5 µL DNA template
ermA	Macrolides	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190		
tetK	Tetracyclines	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360		
ermB	Macrolides	F: CCGTTTACGAAATTGGAACAGGTAAAGGGC R: GAATCGAGACTTGAGTGTGC	359		
grlA	Fluoroquinolones	F: ACTTGAAGATGTTTTAGGTGAT R: TTAGGAAATCTTGATGGCAA	618		
tetM	Tetracyclines	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	1 cycle: 94 ^{0C} ——– 6 min. 34 cycles: 95 ^{0C} ——– 50 s 55 ^{0C} ——– 70 s 72 ^{0C} ——– 60 s 1 cycle: 72 ^{0C} ——– 8 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 200 µM dNTP 0.5 µM of each primers F & R 1.5 U Taq DNA polymerase 5 µL DNA template
gyrA	Fluoroquinolones	F: AGTACATCGTCGTATACTATATGG R: ATCACGTAACAGTTCAAGTGTG	280		
msrA	Macrolides	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	1 cycle: 94 ^{0C} ——– 6 min. 34 cycles: 95 ^{0C} ——– 60 s 50 ^{0C} ——– 70 s 72 ^{0C} ——– 70 s 1 cycle: 72 ^{0C} ——– 8 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
msrB	Macrolides	F: TATGATATCCATAATAATTATCCAATC R: AAGTTATATCATGAATAGATTGTCCTGTT	595		
dfrA1	Folate pathway antagonists	F: CTCACGATAAACAAAGAGTCA R: CAATCATTGCTTCGTATAACG	201		
linA	Lincosamides	F: GGTGGCTGGGGGGTAGATGTATTAACTGG R: GCTTCTTTTGAAATACATGGTATTTTTCGA	323	1 cycle: 94 ^{0C} ——– 6 min. 30 cycles: 95 ^{0C} ——– 60 s 57 ^{0C} ——– 60 s	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template

				72 ^{0C} ——— — 60 s 1 cycle: 72 ^{0C} ——— — 10 min	
rpoB	Ansamycins	F: ACCGTCGTTTACGTTCTGTA R: TCAGTGATAGCATGTGTATC	460	1 cycle: 94 ^{0C} ——— – 5 min. 40 cycles: 94 ^{0C} ——— – 40 s 45.5 ^{0C} ——— —— 40 s 72 ^{0C} ——— — 90 s 1 cycle: 72 ^{0C} ——— — 8 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
blaZ	Penicillins	F: TGAACCGTATGTTAGTGC R: GTCGTGTTAGCGTTGATA	681	1 cycle: 94 ^{0C} ——— – 6 min. 30 cycles: 95 ^{0C} ——— – 60 s 59 ^{0C} ——— — 60 s 72 ^{0C} ——— — 60 s 1 cycle: 72 ^{0C} ——— — 10 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
fexA	Phenicols	F: GTACTTGTAGGTGCAATTACGGCTGA R: CGCATCTGAGTAGGACATAGCGTC	1272	1 cycle: 94 ^{0C} ——— – 1 min. 34 cycles: 94 ^{0C} ——— – 1 min 57 ^{0C} ——— — 2 min 72 ^{0C} ——— — 3 min 1 cycle: 72 ^{0C} ——— — 7 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
vanA	Glycopeptides	F: ATGAATAGAATAAAAGTTGC R: TCACCCCTTTAACGCTAATA	1032	1 cycle: 98 ^{0C} ——— – 2 min. 35 cycles:	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP

				98 ^{0C} ——– 10 s 50 ^{0C} ——– 60 s 72 ^{0C} ——– 90 s 1 cycle: 72 ^{0C} ——– 10 min	0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
vanB	Glycopeptides	F: GTGACAAACCGGAGGCGAGGA R: CCGCCATCCTCCTGCAAAAAA	430	1 cycle: 94 ^{0C} ——– 10 min. 30 cycles: 94 ^{0C} ——– 30 s 50 ^{0C} ——– 45 s 72 ^{0C} ——– 30 s 1 cycle: 72 ^{0C} ——– 10 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
mecA	Penicillins	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGCAGTACCGGATTTGC	532	1 cycle: 94 ^{0C} ——– 2 min. 30 cycles: 94 ^{0C} ——– 30 s 55 ^{0C} ——– 30 s 72 ^{0C} ——– 30 s 1 cycle: 72 ^{0C} ——– 4 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
vatA	Streptogramins	F: TGGTCCCGGAACAACATTTAT R: TCCACCGACAATAGAATAGGG	268	1 cycle: 94 ^{0C} ——– 6 min. 34 cycle: 95 ^{0C} ——– 50 s 55 ^{0C} ——– 70 s 72 ^{0C} ——– 60 s 1 cycle: 72 ^{0C} ——– 8 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 200 µM dNTP 0.5 µM of each primers 1.5 U Taq DNA polymerase 5 µL DNA template
vatB	Streptogramins	F: GCTGCGAATTCAGTTGTTACA R: CTGACCAATCCCACCATTTTA	136	1 cycle:	5 µL PCR buffer 10X 2 mM $Mgcl_2$

				94 ^{0C} ——– 6 min. 35 cycle: 95 ^{0C} ——– 50 s 55 ^{0C} ——– 70 s 72 ^{0C} ——– 80 s 1 cycle: 72 ^{0C} ——– 10 min	150 µM dNTP 0.75 µM of each primers 1.5 U Taq DNA polymerase 3 µL DNA template
blaCTX-M[a]	Cephems	F: ATGTGCAGYACCAGTAARGT R: TGGGTRAARTARGTSACCAGA	593	1 cycle: 94 ^{0C} ——– 7 min. 35 cycles: 94 ^{0C} ——– 50 s 50 ^{0C} ——– 40 s 72 ^{0C} ——– 60 s 1 cycle: 72 ^{0C} ——– 5 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template

[a]R is A or G; Y is C or T; S is G or C.

Table 2. Contamination rate of diverse kinds of powdered packaged medicinal plants and bottle herbal distillate samples with the *S. aureus*.

Types of samples		N. samples collected	N. samples contaminated with *S. aureus* (%)
Medicinal plants	*Z. multiflora*	30	2 (6.66)
	S. bachtiarica	30	2 (6.66)
	A. citrodora	30	3 (10)
	R. damascene	30	3 (10)
	Total	**120**	**10 (8.33)**
Herbal Distillates	*L. angustifolia*	30	4 (13.33)
	A. maurorum	30	5 (16.66)
	C. intybus	30	3 (10)
	M. officinalis	30	2 (6.66)
	M. piperita	30	2 (6.66)
	F. officinalis	30	4 (13.33)
	Total	**180**	**20 (11.11)**
Total		**300**	**30 (10)**

Table 3. The phenotypic pattern of antibiotic resistance of *S. aureus* bacteria isolated from diverse powdered packaged medicinal plants and bottle herbal distillate samples.

Types of samples (N. *S. aureus*)		N. *S. aureus* isolates resist to each antibiotic agent (%)										
		Penicillins		Cephems		Glycopeptides	Aminoglycosides	Macrolides		Tetracyclines		Flur
		Ox	P10	Cfx	Cft	Van	Gen	Az	Ert	Tet	Dox	Cip
Medicinal plants	*Z. multiflora* (2)	1 (50)	2 (100)	1 (50)	1 (50)	-	2 (100)	1 (50)	1 (50)	2 (100)	1 (50)	1 (5
	S. bachtiarica (2)	1 (50)	2 (100)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)	1 (5
	A. citrodora (3)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	2 (66.66)	1 (33.33)	1 (33.
	R. damascene (3)	1 (33.33)	3 (100)	1 (33.33)	1 (33.33)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	2 (66.66)	1 (33.33)	1 (33.
	Total (10)	**4 (40)**	**9 (90)**	**4 (40)**	**4 (40)**	**3 (30)**	**7 (70)**	**4 (40)**	**6 (60)**	**8 (80)**	**4 (40)**	**4 (4**
Herbal Distillates	*L. angustifolia* (4)	2 (50)	4 (100)	2 (50)	2 (50)	1 (25)	3 (75)	2 (50)	3 (75)	4 (100)	2 (50)	2 (5
	A. maurorum (5)	2 (40)	4 (80)	3 (60)	3 (60)	2 (40)	5 (100)	2 (40)	4 (80)	5 (100)	2 (40)	3 (6
	C. intybus (3)	2 (66.66)	3 (100)	2 (66.66)	1 (33.33)	1 (33.33)	3 (100)	1 (33.33)	2 (66.66)	2 (66.66)	1 (33.33)	2 (66.
	M. officinalis (2)	2 (100)	2 (100)	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)	2 (100)	2 (100)	1 (50)	1 (5
	M. piperita (2)	1 (50)	2 (100)	1 (50)	1 (50)	1 (50)	2 (100)	1 (50)	1 (50)	2 (100)	1 (50)	1 (5
	F. officinalis (4)	2 (50)	4 (100)	2 (50)	2 (50)	1 (25)	4 (100)	2 (50)	3 (75)	4 (100)	2 (50)	3 (7
	Total (20)	**11 (55)**	**19 (95)**	**11 (55)**	**10 (50)**	**9 (45)**	**19 (95)**	**9 (45)**	**15 (75)**	**19 (95)**	**9 (45)**	**12 (**
Total (30)		**15 (50)**	**28 (93.33)**	**15 (50)**	**14 (46.66)**	**12 (40)**	**26 (86.66)**	**13 (43.33)**	**21 (70)**	**27 (90)**	**13 (43.33)**	**16 (53.**

*Ox: oxacillin (1 µg/disk), P10: penicillin (10 units/disk), Cfx: cefoxitin (30 µg/disk), Cft: ceftaroline (30 µg/disk), Van: vancomycin (5 µg/disk)), Gen: gentamicin (15 µg/disk), Az: azithromycin (15 µg/disk), Ert: erythromycin (15 µg/disk), Tet: tetracycline (30 µg/disk), Dox: doxycycline (30 µg/disk), Cip: ciprofloxacin (5 µg/disk), Lev: levofloxacin (5 µg/disk), Nit: nitrofurantoin (300 µg/disk), Cln: clindamycin (2 µg/disk), Trs: trimethoprim-sulfamethoxazole (1.25/23.75 µg/disk), C30: chloramphenicol (30 µg/disk), Rif: rifampin (5 µg/disk), Qd: quinupristin-dalfopristin (15 µg/disk).

Table 4. The genotypic pattern of antibiotic resistance of *S. aureus* bacteria isolated from diverse kinds of powdered packaged medicinal plants and bottle herbal distillate samples

Types of samples (N. *S. aureus*)		N. *S. aureus* isolates resist to each antibiotic agent (%)										
		Penicillins		Cephems	Glycopeptides		Aminoglycosides	Macrolides				Tetracycl
		MecA	*blaZ*	*blaCTX-M*	*vanA*	*vanB*	*aacA-D*	*ermA*	*ermB*	*msrA*	*msrB*	*tetK*
Medicinal plants	*Z. multiflora* (2)	1 (50)	1 (50)	1 (50)	-	-	1 (50)	1 (50)	-	1 (50)	-	1 (50)
	S. bachtiarica (2)	1 (50)	1 (50)	1 (50)	1 (50)	-	1 (50)	1 (50)	-	1 (50)	-	1 (50)
	A. citrodora (3)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	-	1 (33.33)	1 (33.33)	1 (33.33)	1 (33.33)	-	2 (66.66)
	R. damascene (3)	1 (33.33)	2 (66.66)	1 (33.33)	-	1 (33.33)	1 (33.33)	1 (33.33)	-	1 (33.33)	1 (33.33)	2 (66.66)
	Total (10)	**4 (40)**	**6 (60)**	**4 (40)**	**2 (20)**	**1 (10)**	**4 (40)**	**4 (40)**	**1 (10)**	**4 (40)**	**1 (10)**	**6 (60)**
Herbal Distillates	*L. angustifolia* (4)	2 (50)	3 (75)	1 (25)	1 (25)	1 (25)	2 (50)	2 (50)	-	2 (50)	-	3 (75)
	A. maurorum (5)	2 (40)	3 (60)	2 (40)	2 (40)	1 (20)	2 (40)	2 (40)	1 (20)	2 (40)	-	3 (60)
	C. intybus (3)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	-	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	-	2 (66.66)
	M. officinalis (2)	1 (50)	1 (50)	1 (50)	1 (50)	-	1 (50)	1 (50)	-	1 (50)	1 (50)	1 (50)
	M. piperita (2)	1 (50)	1 (50)	1 (50)	-	-	1 (50)	1 (50)	-	1 (50)	-	1 (50)
	F. officinalis (4)	2 (50)	3 (75)	1 (25)	1 (25)	1 (25)	2 (50)	2 (50)	-	2 (50)	-	2 (50)
	Total (20)	**9 (45)**	**13 (65)**	**7 (35)**	**6 (30)**	**3 (15)**	**9 (45)**	**10 (50)**	**2 (10)**	**9 (45)**	**1 (5)**	**12 (60)**
Total (30)		**13 (43.33)**	**19 (63.33)**	**11 (36.66)**	**8 (26.66)**	**4 (13.33)**	**13 (43.33)**	**14 (46.66)**	**3 (10)**	**13 (43.33)**	**2 (6.66)**	**18 (60)**

Figures

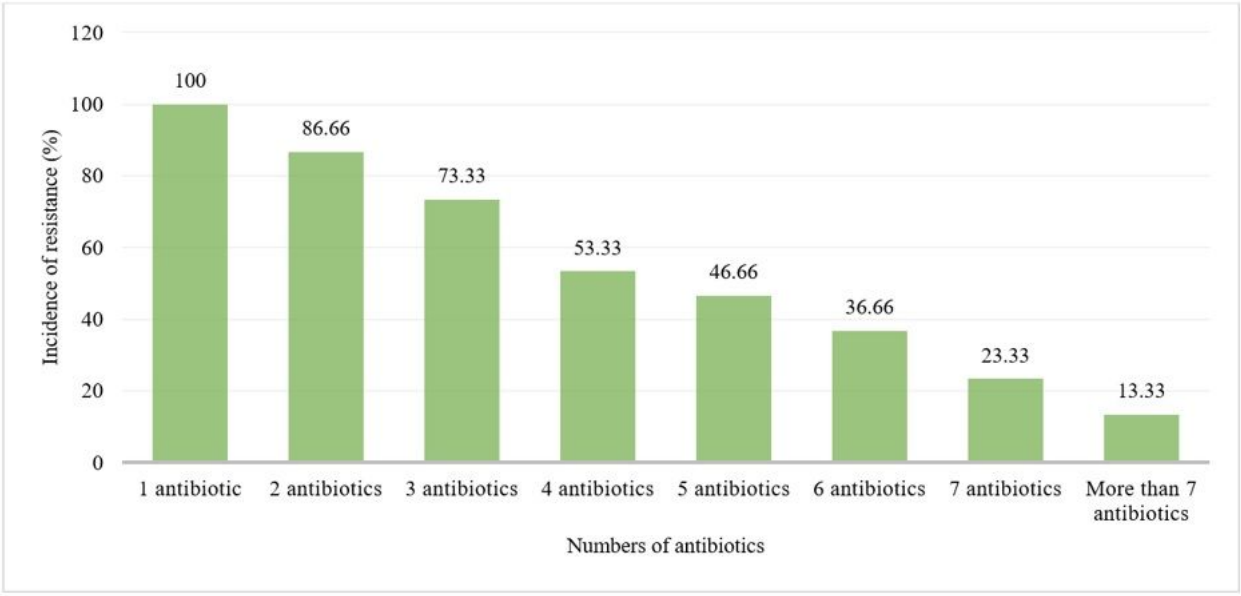

Figure 1

Prevalence of multidrug resistant-S. aureus bacteria isolated from examined powdered packaged medicinal plants and bottle herbal distillate samples. Frequencies were measured according to a total number of 30 S. aureus isolates.

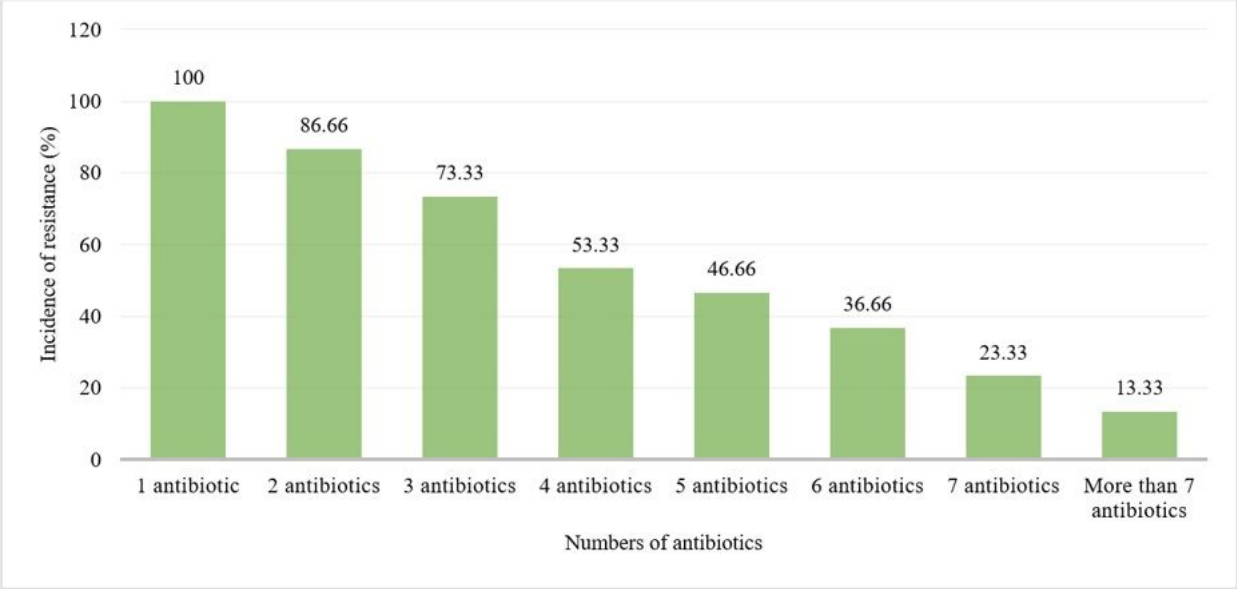

Figure 1

Prevalence of multidrug resistant-S. aureus bacteria isolated from examined powdered packaged medicinal plants and bottle herbal distillate samples. Frequencies were measured according to a total number of 30 S. aureus isolates.