

Prevalence of anterior nares colonization of Palestinian diabetic patients with *Staphylococcus aureus* or methicillin-resistant *Staphylococcus aureus*

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Abstract

Staphylococcus aureus (*S. aureus*) is an opportunistic pathogen that colonizes the anterior nares of about one-third of the human population. Anterior nares colonization with *S. aureus* or methicillin-resistant *S. aureus* (MRSA) allows these pathogens to colonize the skin and other anatomical locations. Accordingly, these pathogens may cause different types of endogenous infections. To investigate the prevalence of nasal carriage of *S. aureus* or MRSA among Palestinian diabetic patients, nasal swabs were taken from 151 diabetic patients, about to undergo invasive surgeries. Thirty-five patients (35.1% of the total patients) were found to be colonized with *S. aureus*, of which 14 (9.7% of the total patients) were found to be colonized with MRSA. These proportions were higher than those described for the general population (30% and 1.3%, respectively) or even for Palestinian patients in general (25.9% and 2%, respectively). In addition, the proportion of nasal carriage of *S. aureus* or MRSA among Palestinian diabetic patients was found to be higher than that described for diabetic patients in other countries. Meanwhile, 30 of the 53 isolates (57% of the total isolates) were also found to be multidrug-resistant. Accordingly, the proportion of anterior nares colonization with *S. aureus* or MRSA in Palestinian diabetic patients was remarkably high.

Keywords: Palestine; diabetes; *S. aureus*; MRSA; antibiotic resistance; anterior nares colonization

Introduction

Staphylococcus aureus (*S. aureus*) is a member of the human microbiota that mainly colonizes the anterior nares of about 30% of the human population (Aswani and Shukla, 2011; Brown *et al.*, 2014, Yang *et al.*, 2022). From the anterior nares, it spreads to various anatomical locations, such as the skin, throat, and vagina (Jauneikaite *et al.*, 2020). As an opportunistic pathogen, *S. aureus* causes community or nosocomial-acquired endogenous infections that range from mild to clinically complicated infections associated with high morbidity and mortality

rates (Alvarez *et al.*, 2010; Hunt *et al.*, 1988; Vandenesch *et al.*, 2003; Wertheim *et al.*, 2005).

This pathogen expresses several surface-adhesion molecules (adhesions) that promote its attachment to several types of host molecules (receptors), such as laminin and fibronectin, fibrin/fibrinogen, and collagen (Sakr *et al.*, 2018). By its anatomical proximity and adhesion molecules, it rapidly colonizes skin wounds by binding to the exposed receptor molecules found on damaged tissues at the wound site (Birkenhauer *et al.*, 2014; Harris *et al.*, 2002). This is the reason for this pathogen to have the

highest proportion of wound-causing infections, compared to other bacterial pathogens (Birkenhauer *et al.*, 2014).

Pathogenic bacteria cause diseases in humans and animals (Hu *et al.*, 2022; Qin *et al.*, 2022; Tian *et al.*, 2022). *S. aureus* causes invasive skin infections ranging from mild ones, such as folliculitis and boils, to severe infections, such as carbuncles (Singer and Talan, 2014). An untreated abscess may enable *S. aureus* and/or its toxic-shock syndrome toxin (TSST) to reach the bloodstream and cause toxic shock, a potentially fatal clinical condition associated with high proportions of mortality and morbidity (Fowler *et al.*, 2003; Lowy, 1998). Once the pathogen reaches the bloodstream, it may get seeded into various tissues and organs, causing serious pyogenic inflammations, such as endocarditis, osteomyelitis, and meningitis (Carek *et al.*, 2001; Fernandez Guerrero *et al.*, 2009; Schlesinger *et al.*, 1987).

In addition, this pathogen is considered as one of the leading bacterial pathogens in terms of its ability to develop resistance to many antibiotics (Mlynarczyk-Bonikowska *et al.*, 2022; Pantosti *et al.*, 2007; Zhang *et al.*, 2023). These mechanisms may include the production of antibiotic-inactivating enzymes via genetic mutations that either alter the target site of an antibiotic or reduce its ability to reach the bacterial cytoplasm and efflux pumps (Mlynarczyk-Bonikowska *et al.*, 2022; Pantosti *et al.*, 2007).

The emergence of certain strains that are highly resistant to antibiotics, such as methicillin-resistant *S. aureus* (MRSA) (Lakhundi and Zhang 2018), and vancomycin-resistant *S. aureus* (VRSA) (McGuinness *et al.*, 2017), has made the treatment of their infections very complicated and thus giving a chance to infections to become severe and life-threatening (Tarai *et al.*, 2013). Interestingly, the global nasal colonization proportion with MRSA was estimated at about 1.3% (Salgado *et al.*, 2003). Initially, MRSA was mainly associated with nosocomial infections. However, since the 1990s, a significant increase in community-acquired MRSA infections has been reported globally (Berman *et al.*, 1993; Gorak *et al.*, 1999; Herold *et al.*, 1998; Pate *et al.*, 1995).

A study conducted by Gould and Cruickshank (1957) demonstrated that about 86% of patients with *S. aureus* skin infections were colonized with the same infecting strain in their anterior nares. Another study conducted in 1990 found a significant link between *S. aureus* nasal colonization and increase in the risk of developing endogenous infections with the same colonizing strain at the exit site of the catheter used in ambulatory peritoneal dialysis (Luzar *et al.*, 1990).

Balanced nutrition has an important role in maintaining adequate immune system that protects us from many microbial infections (Munteanu and Schwartz, 2022). Malnutrition or imbalanced nutrition has a passive effect on the functionality of our immune defenses, and thus rendering us more susceptible to microbial infections as well as development of certain diseases, such as cancer and diabetes (Cui *et al.*, 2020; Foolchand *et al.*, 2022; Gao *et al.*, 2022; Lopez Plaza and Bermejo Lopez, 2017; Saklayen, 2018; Sami *et al.*, 2017).

Hyperglycemia in diabetes has a profound passive effect on immune response to microbial invasion (Berbudi *et al.*, 2020). It has been shown that hyperglycemia interferes with the expression of inflammatory genes of immune cells and thus weakens their inflammatory response to microbial infections (Akbari and Hassan-Zadeh, 2018).

Currently, diabetes is considered as the most common metabolic disorder worldwide, predominantly in low- and middle-income countries (Teufel *et al.*, 2021). Interestingly, a study conducted in 2014 indicated that the global number of diabetic patients is predicted to rise from 382 million in 2013 to 592 million in 2035 (Guariguata *et al.*, 2014). The increase in the prevalence rate of diabetes, mainly in low- and middle-income countries, demands upgrading of intervening procedures that target diabetic patients and may mitigate proportion of both infections and colonization by these pathogens (Stacey *et al.*, 2019).

Many studies have shown that nasal colonization with *S. aureus* or MRSA is higher in diabetic patients, compared to healthy individuals. In 2017, a study conducted in China found that about 8.7% enrolled diabetic patients were colonized with *S. aureus* and 4.1% were colonized by MRSA (Lin *et al.*, 2017). Another study conducted in Turkey in 2006 found that about 35.3% type I and 13.8% type II diabetes enrolled patients were colonized with *S. aureus* (Tamer *et al.*, 2006). Furthermore, it was found that 2.6% diabetics in Japan and 2.1% diabetics across nine European countries were colonized with MRSA (Stacey *et al.*, 2019). It was shown that *S. aureus* or MRSA nasal carriage increases the risk of developing diabetic foot infection among diabetic patients (Dawaiwala *et al.*, 2021; Lavery *et al.*, 2014; Lin *et al.*, 2020). Interestingly, nasal decolonization of these pathogens decreases the risk of developing endogenous infections with these pathogens (Lin *et al.*, 2020; Ontario, 2022; Patel *et al.*, 2022).

No previous study has investigated the proportion of nasal colonization with *S. aureus* or MRSA among diabetic patients in Palestine. The main aim of this study was

to investigate this issue in diabetic patients from the northern part of West-Bank, Palestine, who were about to undergo invasive surgery, and to investigate the antibiotic resistance profiles of colonizing strains.

Materials and Methods

Ethical considerations

Ethical standards and the Declaration of Helsinki were followed while conducting the present study. Institutional Review Board (IRB) committee of the An-Najah National University (ANNU) approved the study (16-10-21). All participants read and declared their consent following relevant guidelines. Written informed consent was obtained from the participants before data collection. Patients included in the study had the right to terminate their involvement at any time during the study, although none of the patients included in the study was forced to do so. In addition, identity of the patients was confidential, and the collected information of each patient was allocated a code number instead of any personal identifying details.

Sampling technique

Nutrient agar, nutrient broth, mannitol sal agar, Mueller Hinton agar, and antibiotic disks were obtained from Oxoid (Basingstoke, Hampshire, UK). Coagulase test was conducted using the Staphylase test kit (Oxoid) according to the manufacturer's instructions.

Nasal swabs from the anterior nares of 151 diabetic patients who were to undergo invasive surgery at Rafidya Surgical Hospital in Nablus City, in the northern part of West Bank, Palestine, were obtained between November 2021 and July 2022.

Each swab was inoculated into a tube containing 4-mL nutrient broth supplemented with 7.5% sodium chloride (NaCl). The inoculated tubes were then incubated in a shaker incubator at 37°C under aerobic conditions for 24 h. By the end of the incubation period, each tube was used to inoculate mannitol salt agar plate using the four-quadrant streaking method to obtain separate colonies. After that, the inoculated plates were incubated at 37°C under aerobic conditions for 24 h. Then, a single yellowish colony (mannitol-fermenting colony) was picked up using a sterile needle and sub-cultured on a nutrient agar plate. The inoculated nutrient agar plates were incubated at 37°C under aerobic conditions for 24 h. After that, the bacteria grown on each plate were harvested using a sterile cotton swab and suspended in 1-mL

nutrient with 40% glycerol using a 2-mL storage cryo-tube. The cryo-tubes were vigorously vortexed and stored at -80°C for the future use (Howard, 1956).

Bacterial Diagnosis of *S. aureus* isolates

The content of each of the frozen cryo-tubes was allowed to thaw at room temperature. Then, each of these tubes was used to inoculate nutrient agar plate. After that, the inoculated plates were incubated at 37°C under aerobic conditions for 24 h.

The grown bacteria were identified as *S. aureus* based on standard microbiological tests (Patricia, 2017). *S. aureus* American Type Culture Collection (ATCC) 25923 and a previously confirmed MRSA clinical isolate were used as controls (Adwan, 2014).

Antibiotics susceptibility testing

All of the obtained *S. aureus* isolates were tested for antibiotic susceptibility to bacitracin (10 µg), cefoxitin (30 µg), clindamycin (2 µg), erythromycin (15 µg), sulfamethoxazole/trimethoprim (1.25/23.73 µg), linezolid (30 µg), teicoplanin (30 µg), vancomycin (30 µg), tetracycline (30 µg), levofloxacin (5 µg), rifampicin (5 µg), azithromycin (15 µg), gentamicin (10 µg), oxacillin (1 µg), and chloramphenicol (30 µg) using the disk-diffusion method based on the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2020).

Briefly, a suspension of each of the obtained isolate was prepared in normal saline at a concentration of 0.5 McFarland (1.5×10^8 CFU/mL). After that, each of the bacterial suspension was used to inoculate three Mueller Hinton agar plates by using a sterile cotton swab. Then by using a disk dispenser, antibiotic disks were placed on the surfaces of inoculated agar plates. After that, the plates were incubated at 35°C for 18 h under aerobic condition. By the end of the incubation period, inhibitions zones around antibiotic disks were measured and used to determine whether the tested stain was susceptible, intermediate-resistant, or resistant to each of the tested antibiotics.

Each of the obtained *S. aureus* isolates was sub-cultured on a nutrient agar plate and incubated at 37°C for 24 h under aerobic conditions. After that, a bacterial suspension of 0.5 McFarland was prepared from each of the grown isolates. Each bacterial suspension was used to inoculate two Mueller Hinton agar plates using a sterile cotton swab. The discs of the above-mentioned antibiotics were distributed evenly on the surfaces of the inoculated plates of each isolate. Then the plates were

incubated at 35°C for 18 h under aerobic conditions. After that, the inhibition zone around each of the used antibiotic discs was measured in millimeter, and each of the obtained isolates was determined as susceptible, intermediate-resistant, or resistant to each antibiotic based on the CLSI (2020) guidelines.

Identification of MRSA in the obtained isolates

MRSA was identified in the obtained *S. aureus* isolates based on cefoxitin resistance as recommended by CLSI (2020) guidelines.

Results and Discussion

Proportion of *S. aureus* and MRSA anterior nares colonization in diabetic patients

Diabetes is a global health problem with several clinical complications significantly affecting global mortality (van der Berg *et al.*, 2016; Zheng *et al.*, 2018; Zimmet *et al.*, 2014). In the Middle East, about 46-million patients had type 2 diabetes between 2000 and 2018 (Kalan Farmanfarma *et al.*, 2020).

In Palestine, the proportion of diabetes mellitus in 2015 was estimated to be about 18.4%. However, this proportion was expected to become 21.5% by 2030 (Abu-Rmeilehi *et al.*, 2013). More importantly, diabetes in Palestine was expected to grow as one of the leading causes of morbidity and mortality, compared to other Middle East countries, indicating that diabetes and its potential complications, including the growth of microbial infections, is becoming a major health problem (Husseini *et al.*, 2009, Rahim *et al.* 2014). A possible explanation for increase in the prevalence of diabetes among Palestinians could be attributed to increase in the proportion of consumption of food and drinks high in fat and sugar as well as decrease in the pattern of physical activities (Al Sabbah *et al.*, 2007; Mikki *et al.*, 2010).

As mentioned earlier, the main goal of this quantitative descriptive cross-sectional study was to determine the prevalence of nasal colonization with *S. aureus* or MRSA among diabetic patients who were to undergo invasive surgeries. To achieve this objective, initially, nasal swabs from 151 patients (mean age 54.85 ± 6.30 years), including 86 (57%) males and 65 (43%) females, with type II diabetes were obtained (Table 1). Of the 151 patients, 53 (35.1%) were found to be colonized with *S. aureus* in the anterior nares.

Methicillin-resistant *S. aureus* was identified among the obtained isolates based on susceptibility to cefoxitin

Table 1. Number, gender, and mean age of the patients.

Total patients	Number (%)	Mean age (±SD)
	151 (100%)	54.8 (6.5)
Males	86 (57%)	52.3 (6.6)
Females	65 (43%)	55.6 (5.4)

(30 µg) as recommended by CLSI (2020). Identification of MRSA based on cefoxitin resistance is shown to have 100% sensitivity and 100% specificity, compared to defecation in *mecA* gene by Polymerase chain reaction (PCR) (Koupahi *et al.*, 2016). Among the obtained 53 *S. aureus* isolates, 14 (9.7% of total patients) were found as MRSA isolates.

Although some studies reported no significant difference in the proportion of *S. aureus* nasal colonization between diabetic patients and healthy individuals (Essigmann *et al.*, 2022), many studies reported that diabetes mellitus is one risk factor that increases the possibility of anterior nares colonization with *S. aureus* (Lin *et al.*, 2017; Stacey *et al.*, 2019; Tamer *et al.*, 2006). Increase in the prevalence rate of *S. aureus* anterior nares colonization could be explained based on the negative impact of diabetes on host immune defenses, particularly innate immunity (Geerlings and Hoepelman, 1999; Lipsky *et al.*, 1987; Sakr *et al.*, 2018). In addition, it is established that increase in glycosylated hemoglobin (HbA1c) level in diabetic patients significantly increases the proportion of *S. aureus* anterior nares colonization, and oral hypoglycemic agents decrease this proportionality (Lin *et al.*, 2020).

The results of the current study revealed that the proportion of *S. aureus* and MRSA nasal colonization among diabetic patients at the time of their admission was about 35.1% and 9.7%, respectively. These proportions were higher than the general proportions of nasal colonization with *S. aureus* and MRSA obtained by a cross-sectional study conducted in Palestine in 2009 (Kaibni *et al.*, 2009). In the cited study, it was found that at the time of hospitalization, about 25.9% and 2% of 834 patients were nasally colonized with *S. aureus* and MRSA, respectively (Kaibni *et al.*, 2009).

In addition, our results indicated that the proportion of nasal colonization with *S. aureus* (35.1%) was higher than the proportion (approximately 30%) reported in the general population (Aswani and Shukla 2011; Brown *et al.*, 2014). This result was supported by other studies showing that diabetes increases *S. aureus* anterior nares colonization (Kluytmans *et al.*, 1997; Tamer *et al.*, 2006).

Furthermore, the higher proportions of *S. aureus* and MRSA nasal colonization found in the present study among diabetic patients (35.1% and 9.7%, respectively)

were higher than those reported for diabetic patients in other countries. A study conducted in China showed that 8.7% and 4.1% of enrolled diabetic patients were colonized with *S. aureus* and MRSA, respectively (Lin et al., 2017). Another study conducted in Ghana reported that 31.0% and 3.3% of diabetic patients were colonized with *S. aureus* and MRSA, respectively (Anafo et al., 2021). In addition, a meta-analysis study comprising both diabetic inpatients and outpatients from different countries and regions (East Asia, the Middle East, Germany, Taiwan, and the United States) established that the proportions of anterior nares colonization with *S. aureus* and MRSA were 13.46% and 8.33%, respectively (Stacey et al., 2019). In this study, we observed a lower proportion of anterior nares colonization among diabetic patients (9.7%) than the one reported by an earlier meta-analysis study (Stacey et al., 2019). However, the proportion obtained in our study was higher than the proportion of MRSA anterior nares colonization among diabetic outpatients (8.33%) reported by the cited study.

The high proportion of MRSA nasal colonization in diabetic patients reported by our study (9.7%) reflected that prescription and utilization of antibiotics were greatly misused in Palestine (Abu Taha et al., 2016; Zyoud et al., 2015).

Antibiotic susceptibility profiles of obtained isolates

The antibiotic susceptibility profiles of 15 antibiotics were examined for each of the obtained isolates using the disc diffusion method (Table 2 and Figure 1). The highest

proportion of resistance was observed for bacitracin, azithromycin, and erythromycin, which was 33 (62.3 %), 26 (49%), and 21 (39.6%) of the obtained isolates, respectively. On the other hand, the lowest proportion of resistance was observed for rifampicin and linezolid, being 2 (3.8%) of the obtained isolates for each of them. Meanwhile, none of the obtained isolates resisted teicoplanin and vancomycin (Table 2 and Figure 1).

Table 2. Number (%) of the obtained *S. aureus* isolates that were resistant (R), intermediate-resistant (IR), or susceptible (S) to each of the used antibiotics.

Antibiotic	R Number (%)	IR Number (%)	S Number (%)
Bacitracin	33 (62.3)	17 (32.1)	3 (5.7)
Azithromycin	26 (49)	11 (20.8)	16 (30.2)
Erythromycin	21 (39.6)	13 (24.5)	19 (35.9)
Gentamicin	18 (34)	4 (7.5)	31 (58.5)
Levofloxacin	15 (28.3)	4 (7.6)	43 (64.1)
Cefoxitin	14 (26.4)	0 (0)	39 (73.6)
Sulfa/trimethoprim	11 (20.8)	0 (0)	42 (79.2)
Oxacillin	10 (18.5)	3 (5.5)	40 (75.5)
Tetracycline	6 (11.3)	1 (1.9)	46 (86.8)
Clindamycin	5 (9.4)	4 (7.6)	44 (83)
Chloramphenicol	5 (9.4)	1 (1.9)	47 (88.7)
Rifampicin	2 (3.8)	0 (0)	51 (96.2)
Linezolid	2 (3.8)	0 (0)	51 (96.2)
Teicoplanin	0 (0)	0 (0)	53 (100)
Vancomycin	0 (0)	0 (0)	53 (100)

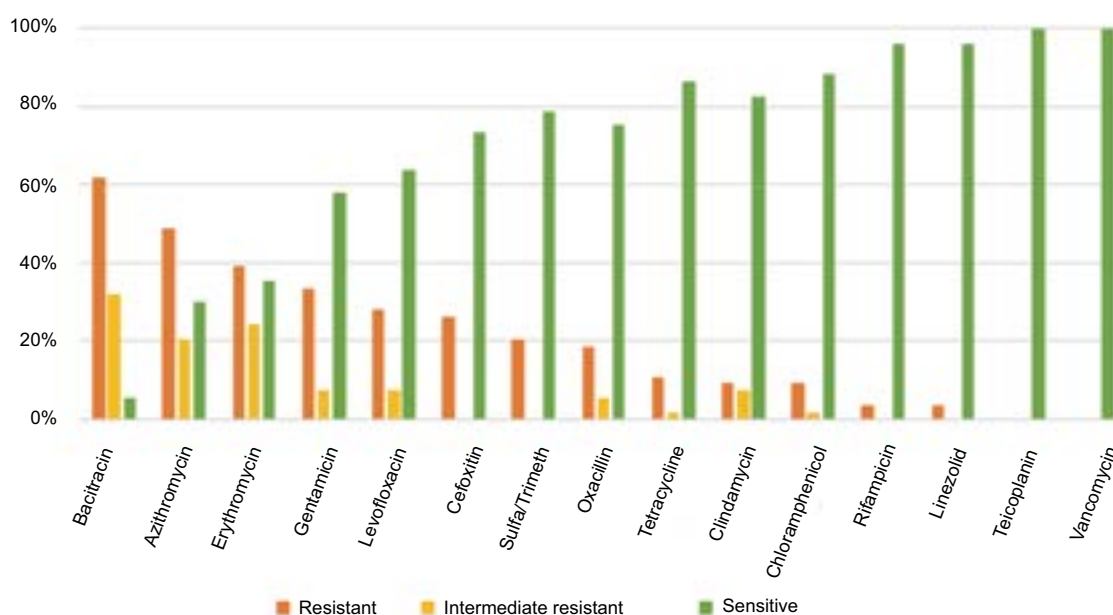


Figure 1. The prevalence proportion of antibiotics susceptibility profiles of the obtained isolates.

Table 3. Proportion of resistance of the obtained *S. aureus* isolates to some of the utilized antibiotics, compared to the proportion of resistance of the same antibiotics by similar isolates in other countries.

Antibiotic	Proportion of resistance (%) of <i>S. aureus</i> isolates to certain antibiotics in other countries		
	Palestine	Italy	Europe
Erythromycin	39.62	34.7	16.5
Gentamicin	33.96	8.4	2.2
Oxacillin	18.87	3.2	–
Fluoroquinolones	28.3	8.4	5.2
Cefoxitin	26.4	3.3	–
Sulfa/trimethoprim	20.75	1.1	1.9
Linezolid	3.77	22.1	–
Tetracycline	11.32	2.8	3.0

Bacitracin is a polypeptide antibiotic used to prepare ointments commonly prescribed globally to treat minor skin injuries, such as cuts, scrapes, and burns (Nguyen *et al.*, 2022). This could explain the high prevalence of resistance among the *S. aureus* isolates obtained in our study (62.3%) and other studies (Nguyen *et al.*, 2022).

Many studies have reported the isolation of teicoplanin- and/or vancomycin-resistant *S. aureus* from clinical samples or anterior nares swabs (Banerjee and Anupurba, 2012; El Sayed *et al.*, 2018; Sujatha and Praharaj, 2012; Szymanek-Majchrzak *et al.*, 2018). The fact that none of the isolates obtained in the present study showed resistance to any of these two antibiotics (see Table 2 and Figure 1) could be due to the rare use of these two antibiotics in Palestine.

Table 3 shows the resistance proportion of the obtained *S. aureus* isolates to some of the antibiotics utilized in this study, compared to the proportion of resistance of the same antibiotics by *S. aureus* isolates obtained by the studies conducted in other countries.

Table 3 clearly shows that, except for the proportion of resistance to linezolid, proportion of resistance to other antibiotics was higher than those of the same antibiotics exhibited by *S. aureus* isolates obtained in Italy (Mascaro *et al.*, 2019). Interestingly, the proportion of resistance of isolates obtained in our study to erythromycin (39.62%), gentamicin (33.96%), fluoroquinolones (28.3%), sulfamethoxazole/trimethoprim (20.75%), and tetracycline (11.32%) was higher than the proportion of resistance to the same antibiotics for isolates obtained in Europe (16.5%, 2.2%, 5.2%, 1.9%, and 3.0%, respectively; Table 2 and Figure 1).

The misuse of prescription and utilization of antibiotics is not only a driving force for the emergence and spread of MRSA in community but also a driving force for the

Table 4. Number (%) of *S. aureus* isolates susceptible to or resistant to one or more of the used antibiotics.

Number (%) of isolates	Number of antibiotics to which the isolates were resistant
3 (5.7%)	No antibiotic
10 (18.9%)	1 antibiotic
10 (18.9%)	2 antibiotics
12 (22.6%)	3 antibiotics
6 (11.3%)	4 antibiotics
3 (5.7%)	5 antibiotics
2 (3.8%)	6 antibiotics
4 (7.5%)	7 antibiotics
2 (3.8%)	8 antibiotics
1 (1.9%)	9 antibiotics

development and spread of multidrug resistant bacteria pathogens (Medina and Pieper, 2016; Ventola, 2015). A multidrug bacterial pathogen is defined as the pathogen that is resistant to at least one antibiotic of three or four categories of antibiotics (Magiorakos *et al.*, 2012).

Although our results showed that 3 (5.7%) of the obtained isolates were susceptible to all of the used antibiotics, 20 (37.8%) of the obtained isolates were resistant to 1–2 of the used antibiotics, 18 (33.9%) of the obtained isolates were resistant to 3–4 of the used antibiotics, 5 (9.5%) of the obtained isolates were resistant to 5–6 of the used antibiotics, 6 (11.3%) of the obtained isolates were resistant to 7–8 of the used antibiotics, and only 1 (1.9%) of the obtained isolates was resistant to 9 of the used antibiotics (Table 4).

Our results indicated that 30 (57%) of our isolates were multidrug-resistant isolates (Table 4), a percentage that could be considered seriously alarming, because such multidrug-resistant isolates could cause endogenous infections that are clinically challenging in terms of their treatment.

The data presented in Tables 2 and 3 and Figure 1 clearly show that the prevalence of antibiotic resistance of *S. aureus* isolates in Palestine has reached alarming proportions, which reflect, as mentioned earlier, the misuse of prescription and utilization of antibiotics in Palestine (Abu Taha *et al.*, 2016; Zyoud *et al.*, 2015).

S. aureus can cause various types of infections (Lowy, 1998). Interestingly, most of these infections are endogenous, caused by the same strains that colonize the anterior nares of patients (Wertheim *et al.*, 2005). The fact that about 57% of the obtained *S. aureus* isolates in the current study were multidrug-resistant isolates (see Table 4), endogenous infections caused by these could be clinically complicated and life-threatening.

Conclusion

Our results indicated that about 35.1% of the enrolled patients in this study were colonized with *S. aureus*, of which 23% were colonized with MRSA, and more than half (57%) of the isolates were multidrug-resistant isolates. Importantly, our findings elucidated the importance of screening of Palestinian diabetic patients for *S. aureus* or MRSA nasal colonization who were to undergo invasive surgery and implementing a decolonization plan to minimize the risk of developing endogenous surgical wound infections or other infections.

A relatively small number of enrolled patients were one of the limitations of this study. In addition, our experimental procedures did not include molecular methods that could be used to confirm the genetic basis of antibiotic resistance.

Availability of data and materials

The datasets used and/or analyzed in the study are available from the corresponding author upon reasonable request.

Author contributions

The study was designed by Muna M. Abbas, Motasim Almasri, and Alaeddin Abu-Zant. Data were analyzed by Shadi Sharef and Sara Mahajne. Manuscript was prepared by Muna M. Abbas, Alaeddin Abu-Zant, Motasim Almasri, and Khalil Kananbi.

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References

Abu-Rmeileh, N.M., Husseini, A., Capewell, S. and O'Flaherty, M. 2013. Preventing type 2 diabetes among Palestinians: comparing five future policy scenarios. *BMJ Open* 3(12): e003558. <https://doi.org/10.1136/bmjopen-2013-003558>

Abu Taha, A., Abu-Zaydeh, A.H.R.A., Ardah, S.W., Al-Jabi, W.M., Sweileh, R. and Awang, S.H. 2016. Public knowledge and

attitudes regarding the use of antibiotics and resistance: findings from a cross-sectional study among Palestinian adults. *Zoonoses Public Health* 63(6): 449–457. <https://doi.org/10.1111/zph.12249>

Adwan, K. 2014. Fast DNA isolation and PCR protocols for detection of methicillin-resistant staphylococci. *Folia Microbiologica (Praha)* 59(1): 5–8. <https://doi.org/10.1007/s12223-013-0259-1>

Akbari, M. and Hassan-Zadeh, V. 2018. Hyperglycemia affects the expression of inflammatory genes in peripheral blood mononuclear cells of patients with type 2 diabetes. *Immunological Investigations* 47(7): 654–665. <https://doi.org/10.1080/08820139.2018.1480031>

Al Sabbah, H., Vereecken, C., Kolsteren, P., Abdeen, Z. and Maes, L. 2007. Food habits and physical activity patterns among Palestinian adolescents: findings from the national study of Palestinian school-children (HBSC-WBG2004). *Public Health Nutrition* 10(7): 739–746. <https://doi.org/10.1017/S1368980007665501>

Alvarez, C.A., Yomayusa, N., Leal, A.L., Moreno, J., Mendez-Alvarez, S., Ibañez, M. and Vanegas, N. 2010. Nosocomial infections caused by community-associated methicillin-resistant *Staphylococcus aureus* in Colombia. *American Journal of Infection Control* 38(4): 315–318. <https://doi.org/10.1016/j.ajic.2009.05.013>

Anafo, R.B., Atiase, Y., Kotey, F.C.N., Dayie, N.T.K.D., Tetteh-Quarcoop, P.B., Duodu, S. et al. 2021. Methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage among patients with diabetes at the Korle Bu Teaching Hospital. *PLoS One* 16(9): e0257004. <https://doi.org/10.1371/journal.pone.0257004>

Aswani, V.H. and Shukla, S.K. 2011. Prevalence of *Staphylococcus aureus* and lack of its lytic bacteriophages in the anterior nares of patients and healthcare workers at a rural clinic. *Clinical Medical Research* 9(2): 75–81. <https://doi.org/10.3121/cm.2010.954>

Banerjee, T. and Anupurba, S. 2012. Colonization with vancomycin-intermediate *Staphylococcus aureus* strains containing the *vanA* resistance gene in a tertiary-care center in north India. *Journal of Clinical Microbiology* 50(5): 1730–1732. <https://doi.org/10.1128/JCM.06208-11>

Berbudi, A., Rahmadika, N., Tjahjadi, A.I. and Ruslami, R. 2020. Type 2 diabetes and its impact on the immune system. *Current Diabetes Review* 16(5): 442–449. <https://doi.org/10.2174/1573399815666191024085838>

Berman, D.S. and Eisner, W. 1993. Community-acquired methicillin-resistant *Staphylococcus aureus* infection. *New England Journal of Medicine* 329(25): 1896. <https://doi.org/10.1056/NEJM199312163292517>

Birkenhauer, E., Neethirajan, S. and Birkenhauer, E. 2014. Collagen and hyaluronan at wound sites influence early polymicrobial biofilm adhesive events. *BMC Microbiology* 14: 191. <https://doi.org/10.1186/1471-2180-14-191>

Brown, A.F., Leech, J.M. and Brown, A.F. 2014. *Staphylococcus aureus* colonization: modulation of host immune response and impact on human vaccine design. *Frontiers in Immunology* 4: 507. <https://doi.org/10.3389/fimmu.2013.00507>

Carek, P.J., Dickerson, L.M. and Sack, J.L. 2001. Diagnosis and management of osteomyelitis. *American Family Physician* 63(12): 2413–2420.

Clinical Laboratory Standards Institute (CLSI). 2020. Performance standards for antimicrobial susceptibility testing. Available

- at: <https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf> (Accessed February, 2021).
- Cui, G., Zhao, K., You, K., Gao, Z., Kakuchi, T., Feng, B. and Duan, Q. 2020. Synthesis and characterization of phenylboronic acid-containing polymer for glucose-triggered drug delivery+. *Science and Technology of Advanced Materials* 21(1): 1–10. <https://doi.org/10.1080/14686996.2019.1700394>
- Dawaiwala, I., Awaghade, S., Kolhatkar, P., Pawar, S. and Barsode, S. 2021. Microbiological pattern, antimicrobial resistance and prevalence of MDR/XDR organisms in patients with diabetic foot infection in an Indian tertiary care hospital. *International Journal of Lower Extremity Wounds* 12(3): 47–53. <https://doi.org/10.1177/15347346211038090>
- El Sayed, N., Ashour, M., and Amine, A.E.K. 2018. Vancomycin resistance among *Staphylococcus aureus* isolates in a rural setting, Egypt. *Germs* 8(3): 134–139. <https://doi.org/10.18683/germs.2018.1140>
- Essigmann, H.T., Hanis, C.L., DeSantis, S.M., Perkison, W.B., Aguilar, D.A., Jun, G.D. and Robinson, A. and Brown, E.L. 2022. Worsening glycemia increases the odds of intermittent but not persistent *Staphylococcus aureus* nasal carriage in two cohorts of Mexican-American adults. *Microbiology Spectrum* 10(3): e0000922. <https://doi.org/10.1128/spectrum.00009-22>
- Fernández Guerrero, M.L., González López, J.J., Goyenechea, A., Fraile, J. and de Górgolas, M. 2009. Endocarditis caused by *Staphylococcus aureus*: a reappraisal of the epidemiologic, clinical, and pathologic manifestations with analysis of factors determining outcome. *Medicine (Baltimore)* 88(1): 1–22. <https://doi.org/10.1097/MD.0b013e318194da65>
- Foolchand, A. and Ghazi, T. 2022. Malnutrition and dietary habits alter the immune system which may consequently influence SARS-CoV-2 virulence: a review. *International Journal of Molecular Sciences* 23(5): 46–53. <https://doi.org/10.3390/ijms23052654>
- Fowler, V.G., Olsen, M.K. and Corey, G.R. 2003. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. *Archives of Internal Medicine* 163(17): 2066–2072. <https://doi.org/10.1001/archinte.163.17.2066>
- Gao, G., Pan, X., Shao, J., Jiang, X., Su, Z., Jin, K. and Ye, J. 2022. Automatic interpretation and clinical evaluation for fundus fluorescein angiography images of diabetic retinopathy patients by deep learning. *British Journal of Ophthalmology* 15(2): 36–46. <https://doi.org/10.1136/bjo-2022-321472>
- Geerlings, S.E. and Hoepelman, A.I. 1999. Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunology and Medical Microbiology* 26(3–4): 259–265. <https://doi.org/10.1111/j.1574-695X.1999.tb01397.x>
- Gorak, E.J., Yamada, S.M. and Brown, J.D. 1999. Community-acquired methicillin-resistant *Staphylococcus aureus* in hospitalized adults and children without known risk factors. *Clinical Infectious Diseases* 29(4): 797–800. <https://doi.org/10.1086/520437>
- Gould, J.C. and Cruikshank, J.D. 1957. Staphylococcal infection in general practice. *Lancet* 273(7006): 1157–1161. [https://doi.org/10.1016/S0140-6736\(57\)92063-9](https://doi.org/10.1016/S0140-6736(57)92063-9)
- Guariguata, L., Whiting, D.R., Hambleton, I., Beagley, J., Linnenkamp, U. and Shaw, J.E. 2014. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice* 103(2): 137–149. <https://doi.org/10.1016/j.diabres.2013.11.002>
- Harris, L.G., Foster, S.J. and Richards, R.G. 2002. An introduction to *Staphylococcus aureus*, and techniques for identifying and quantifying *S. aureus* adhesins in relation to adhesion to biomaterials: review. *European Cells & Materials* 4: 39–60. <https://doi.org/10.22203/ecm.v004a04>
- Herold, B.C. Immergluck, L.C. and Maranan, M.C. 1998. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *Journal of American Medical Association (JAMA)* 279(8): 593–598. <https://doi.org/10.1001/jama.279.8.593>
- Howard, D.H. 1956. The preservation of bacteria by freezing in glycerol broth. *Journal of Bacteriology* 71(5): 625. <https://doi.org/10.1128/jb.71.5.625-625.1956>
- Hu, B., Das, P., Lv, D., Shi, M., Aa, J., Wang, K. et al. 2022. Effects of healthy fecal microbiota transplantation against the deterioration of depression in fawn-hooded rats. *Msystems* 7(3): e00218–e00222. <https://doi.org/10.1128/msystems.00218-22>
- Hunt, J.L., Purdue, G.F. and Tuggle, D.W. 1988. Morbidity and mortality of an endemic pathogen: methicillin-resistant *Staphylococcus aureus*. *American Journal of Surgery* 156(6): 524–528. [https://doi.org/10.1016/S0002-9610\(88\)80545-2](https://doi.org/10.1016/S0002-9610(88)80545-2)
- Husseini, A., Abu-Rmeileh, N.M.E., Mikki, N. and Ramahi, T.M. 2009. Cardiovascular diseases, diabetes mellitus, and cancer in the occupied Palestinian territory. *Lancet* 373(9668): 1041–1049. [https://doi.org/10.1016/S0140-6736\(09\)60109-4](https://doi.org/10.1016/S0140-6736(09)60109-4)
- Jauneikaite, E., Ferguson, T., Mosavie, M., Fallowfield, J.L., Davey, T., Thorpe, N. et al. 2020. *Staphylococcus aureus* colonization and acquisition of skin and soft tissue infection among Royal Marines recruits: a prospective cohort study. *Clinical Microbiology and Infection* 26(3): 381 e381–381 e386. <https://doi.org/10.1016/j.cmi.2019.07.014>
- Kaibni, M.H., Farraj, M.A., Adwan, K. and Essawi, T.A. 2009. Community-acquired methicillin-resistant *Staphylococcus aureus* in Palestine. *Journal of Medical Microbiology* 58(Pt 5): 644–647. <https://doi.org/10.1099/jmm.0.007617-0>
- Kalan Farmanfarma, K.H., Ansari-Moghaddam, A. Zareban, I. and Adineh, H.A. 2020. Prevalence of type 2 diabetes in Middle-East: systematic review & meta-analysis. *Primary Care Diabetes* 14(4): 297–304. <https://doi.org/10.1016/j.pcd.2020.01.003>
- Kluytmans, J. van Belkum, A. and Verbrugh, H. 1997. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clinical Microbiology Reviews* 10(3): 505–520. <https://doi.org/10.1128/CMR.10.3.505>
- Koupahi, H., Jahromy, S.H. and Rahbar, M. 2016. Evaluation of different phenotypic and genotypic methods for detection of methicillin-resistant *Staphylococcus aureus* (MRSA). *Iranian Journal of Pathology* 11(4): 370–376.
- Lakhundi, S. and Zhang, K. 2018. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. *Clinical Microbiology Reviews* 31(4): 25–36. <https://doi.org/10.1128/CMR.00020-18>
- Lavery, L.A., La Fontaine, J., Bhavan, K., Kim, P.J., Williams, J.R. and Hunt, N.A. 2014. Risk factors for methicillin-resistant

- Staphylococcus aureus in diabetic foot infections. *Diabetic Foot & Ankle* 5(1): 25–36. <https://doi.org/10.3402/dfa.v5.23575>
- Lin, J., Xu, P., Peng, Y., Lin, D., Ou, D., Zhang, T., Bai, C. et al. 2017. Prevalence and characteristics of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus nasal colonization among a community-based diabetes population in Foshan, China. *Journal of Diabetes Investigation* 8(3): 383–391. <https://doi.org/10.1111/jdi.12591>
- Lin, S-Y., Lin, N-Y., Huang, Y-Y., Hsieh, C-C. and Huang, Y-C. 2020. Methicillin-resistant Staphylococcus aureus nasal carriage and infection among patients with diabetic foot ulcer. *Journal of Microbiology, Immunology and Infection* 53(2): 292–299. <https://doi.org/10.1016/j.jmii.2018.03.005>
- Lipsky, B.A., Pecoraro, R.E., Chen, M.S. and Koepsell, T.D. 1987. Factors affecting staphylococcal colonization among NIDDM outpatients. *Diabetes Care* 10(4): 483–486. <https://doi.org/10.2337/diacare.10.4.483>
- Lopez Plaza, B. and Bermejo Lopez, L.M. 2017. Nutrition and immune system disorders. *Nutrición Hospitalaria* 34(Suppl 4): 68–71. <https://doi.org/10.20960/nh.1575>
- Lowy, F.D. 1998. Staphylococcus aureus infections. *New England Journal of Medicine* 339(8): 520–532. <https://doi.org/10.1056/NEJM199808203390806>
- Coles, G.A., Faller, B., Slingeneyer, A., Dah, G.A., Briat, C. et al. 1990. Staphylococcus aureus nasal carriage and infection in patients on continuous ambulatory peritoneal dialysis. *New England Journal of Medicine* 322(8): 505–509. <https://doi.org/10.1056/NEJM199002223220804>
- Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G. et al. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* 18(3): 268–281. <https://doi.org/10.1111/j.1469-0691.2011.03570.x>
- Mascaro, V., Squillace, L., Nobile, C.G.A., Papadopoli, R., Bosch, T., Schouls, L.M. et al. 2019. Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) carriage and pattern of antibiotic resistance among sheep farmers from southern Italy. *Infection and Drug Resistance* 12: 2561–2571. <https://doi.org/10.2147/IDR.S211629>
- McGuinness, W.A., Malachowa, N. and DeLeo, F.R. 2017. Vancomycin resistance in Staphylococcus aureus. *Yale Journal of Biology and Medicine* 90(2): 269–281.
- Medina, E. and Pieper, D.H. 2016. Tackling threats and future problems of multidrug-resistant bacteria. *Current Topics in Microbiology and Immunology* 398: 3–33. https://doi.org/10.1007/82_2016_492
- Mikki, N., Abdul-Rahim, H.F., Shi, Z. and Holmboe-Ottesen, G. 2010. Dietary habits of Palestinian adolescents and associated sociodemographic characteristics in Ramallah, Nablus and Hebron governorates. *Public Health Nutrition* 13(9): 1419–1429. <https://doi.org/10.1017/S1368980010000662>
- Młynarczyk-Bonikowska, B., Kowalewski, C., Krolak-Ulinska, A. and Marusza, W. 2022. Molecular mechanisms of drug resistance in Staphylococcus aureus. *International Journal of Molecular Science* 23(15): 8088. <https://doi.org/10.3390/ijms23158088>
- Munteanu, C. and Schwartz, B. 2022. The relationship between nutrition and the immune system. *Frontiers in Nutrition* 9: 1082500. <https://doi.org/10.3389/fnut.2022.1082500>
- Nguyen, R. et al. 2022. Bacitracin topical. StatPearls, Treasure Island, FL.
- Ontario, H. 2022. Pre-surgical nasal decolonization of Staphylococcus aureus: a health technology assessment. *Ontario Health Technology Assessment* 22(4): 1–165.
- Pantosti, A., Sanchini, A. and Monaco, M. 2007. Mechanisms of antibiotic resistance in Staphylococcus aureus. *Future in Microbiology* 2(3): 323–334. <https://doi.org/10.2217/17460913.2.3.323>
- Pate, K.R., Nolah, R.L., Bannerman, T.L. and Feldman, S. 1995. Methicillin-resistant Staphylococcus aureus in the community. *Lancet* 346(8980): 978. [https://doi.org/10.1016/S0140-6736\(95\)91605-9](https://doi.org/10.1016/S0140-6736(95)91605-9)
- Patel, N., Gold, J., Brown, N.J., Abraham, M., Beyer, R.S., Yang, C. et al. 2022. Staphylococcus aureus swabbing and decolonization before neuromodulation procedures: a systematic review and meta-analysis. *Neuromodulation* 26(5): 928–937. <https://doi.org/10.1016/j.neurom.2022.07.013>
- Patricia, M. 2017. *Bailey & Scott's diagnostic microbiology*. Elsevier, St Louis, MO.
- Qin, X., Zhang, K., Fan, Y., Fang, H., Nie, Y. and Wu, X-L. 2022. The bacterial MtrAB two-component system regulates the cell wall homeostasis responding to environmental alkaline stress. *Microbiology Spectrum* 10(5): e02311–e02322. <https://doi.org/10.1128/spectrum.02311-22>
- Rahim, H.F.A., Sibai, A., Khader, Y., Hwalla, N., Fadhil, I., Alsiyabi, H., et al. 2014. Non-communicable diseases in the Arab world. *Lancet* 383(9914): 356–367. [https://doi.org/10.1016/S0140-6736\(13\)62383-1](https://doi.org/10.1016/S0140-6736(13)62383-1)
- Saklayen, M.G. 2018. The global epidemic of the metabolic syndrome. *Current Hypertension Reports* 20(2): 12. <https://doi.org/10.1007/s11906-018-0812-z>
- Sakr, A., Brégeon, F., Mège, J-L., Rolain, J-M., Blin, O. 2018. Staphylococcus aureus nasal colonization: an update on mechanisms, epidemiology, risk factors, and subsequent infections. *Frontiers in Microbiology* 9: 2419. <https://doi.org/10.3389/fmicb.2018.02419>
- Salgado, C.D., Farr, B.M. and Calfee, D.P. 2003. Community-acquired methicillin-resistant Staphylococcus aureus: a meta-analysis of prevalence and risk factors. *Clinical Infectious Diseases* 36(2): 131–139. <https://doi.org/10.1086/345436>
- Sami, W., Ansari, T., Butt, N.S. and Ab Hamid, M.R. 2017. Effect of diet on type 2 diabetes mellitus: a review. *International Journal of Health Sciences (Qassim)* 11(2): 65–71.
- Schlesinger, L.S., Stephen, C.R. and Dennis, R.G. 1987. Staphylococcus aureus meningitis: a broad-based epidemiologic study. *Medicine (Baltimore)* 66(2): 148–156. <https://doi.org/10.1097/00005792-198703000-00006>
- Singer, A.J. and Talan, D.A. 2014. Management of skin abscesses in the era of methicillin-resistant Staphylococcus aureus.

- New England Journal of Medicine 370(11): 1039–1047. <https://doi.org/10.1056/NEJMra1212788>
- Stacey, H.J., Clements, C.S., Welburn, S.C. and Jones, J.D. 2019. The prevalence of methicillin-resistant *Staphylococcus aureus* among diabetic patients: a meta-analysis. *Acta Diabetologica* 56(8): 907–921. <https://doi.org/10.1007/s00592-019-01301-0>
- Sujatha, S. and Praharaj, I. 2012. Glycopeptide resistance in gram-positive cocci: a review. *Interdisciplinary Perspectives on Infectious Diseases* 2012: 781679. <https://doi.org/10.1155/2012/781679>
- Szymanek-Majchrzak, K., Mlynarczyk, A. and Mlynarczyk, G. 2018. Characteristics of glycopeptide-resistant *Staphylococcus aureus* strains isolated from inpatients of three teaching hospitals in Warsaw, Poland. *Antimicrobial Resistance & Infection Control* 7: 105. <https://doi.org/10.1186/s13756-018-0397-y>
- Tamer, A., Karabay, O. and Ekerbicer, H. 2006. *Staphylococcus aureus* nasal carriage and associated factors in type 2 diabetic patients. *Japan Journal of Infectious Diseases* 59(1): 10–14.
- Tarai, B., Das, P. and Kumar, D. 2013. Recurrent challenges for clinicians: emergence of methicillin-resistant *Staphylococcus aureus*, vancomycin resistance, and current treatment options. *Journal of Laboratory Physicians* 5(2): 71–78. <https://doi.org/10.4103/0974-2727.119843>
- Teufel, F., Seiglie, J.A., Geldsetzer, P., Theilmann, M., Marcus, M.E., Ebert, C., Arboleda, W.A.L. et al. 2021. Body-mass index and diabetes risk in 57 low-income and middle-income countries: a cross-sectional study of nationally representative, individual-level data in 685 616 adults. *Lancet* 398(10296): 238–248. [https://doi.org/10.1016/S0140-6736\(21\)00844-8](https://doi.org/10.1016/S0140-6736(21)00844-8)
- Tian, Z., Zhang, Y., Zheng, Z., Zhang, M., Zhang, T., Jin, J. et al. 2022. Gut microbiome dysbiosis contributes to abdominal aortic aneurysm by promoting neutrophil extracellular trap formation. *Cell Host & Microbe* 30(10): 1450–1463. e1458. <https://doi.org/10.1016/j.chom.2022.09.004>
- van der Berg, J.D., Stehouwer, C.D.A., Bosma, H., van der Velde, J.H.P.M., Willems, P.J.B., Savelberg, H.H.C.M. et al. 2016. Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: the Maastricht study. *Diabetologia* 59(4): 709–718. <https://doi.org/10.1007/s00125-015-3861-8>
- Vandenesch, F., Naimi, T., Enright, M.C., Lina, G., Nimmo, G.R., Heffernan, H. et al. 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying panton-valentine leukocidin genes: worldwide emergence. *Emerging Infectious Diseases* 9(8): 978–984. <https://doi.org/10.3201/eid0908.030089>
- Ventola, C.L. 2015. The antibiotic resistance crisis: part 1: causes and threats. *Physical Therapy* 40(4): 277–283.
- Wertheim, H.F., Melles, D.C., Vos, M.C., van Leeuwen, W., van Belkum, A., Verbrugh, H.A., and Nouwen, J.L. 2005. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infectious Diseases* 5(12): 751–762. [https://doi.org/10.1016/S1473-3099\(05\)70295-4](https://doi.org/10.1016/S1473-3099(05)70295-4)
- Yang, R., Hou, E., Cheng, W., Yan, X., Zhang, T., Li, S. et al. 2022. Membrane-targeting neolignan-antimicrobial peptide mimic conjugates to combat methicillin-resistant *Staphylococcus aureus* (MRSA) infections. *Journal of Medicinal Chemistry* 65(24): 16879–16892. <https://doi.org/10.1021/acs.jmedchem.2c01674>
- Zhang, N., Lu, D., Sheng, H., Xia, J., Kan, P., Yao, Z. et al. 2023. Constructed wetlands as hotspots of antibiotic resistance genes and pathogens: evidence from metagenomic analysis in Chinese rural areas. *Journal of Hazardous Materials* 447: 130778. <https://doi.org/10.1016/j.jhazmat.2023.130778>
- Zheng, Y., Ley, S.H. and Hu, F.B. 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology* 14(2): 88–98. <https://doi.org/10.1038/nrendo.2017.151>
- Zimmet, P.Z., Magliano, D.J., Herman, W.H. and Shaw, J.E. 2014. Diabetes: a 21st century challenge. *Lancet Diabetes and Endocrinology* 2(1): 56–64. [https://doi.org/10.1016/S2213-8587\(13\)70112-8](https://doi.org/10.1016/S2213-8587(13)70112-8)
- Zyoud, S.H., Taha, A.A., Araj, K.F., Abahri, I.A., Sawalha, A.F., Sweileh, W.M. et al. 2015. Parental knowledge, attitudes and practices regarding antibiotic use for acute upper respiratory tract infections in children: a cross-sectional study in Palestine. *BMC Pediatrics* 15: 176. <https://doi.org/10.1186/s12887-015-0494-5>