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Antibiotic resistance profiles of *Staphylococcus* spp. from white button mushrooms and handlers

The presence of *Staphylococcus* spp. has increasingly been reported in food products and poses a public health threat. The aim of this study was to determine the diversity of *Staphylococcus* spp. and the antibiotic resistance profiles of isolates obtained from freshly harvested and packed ready-to-eat mushrooms (*n*=432) and handlers' hands (*n*=150). A total of 56 *Staphylococcus* isolates [46.4% (*n*=26) from hands and 53.6% (*n*=30) from mushrooms] were recovered belonging to 10 species. *Staphylococcus succinus* isolates (*n*=21) were the most prevalent, of which 52.4% came from mushrooms and 47.6% from hands. This was followed by *S. equorum* isolates [*n*=12; 91.7% (*n*=11) from mushrooms and 8.3% (*n*=1) from hands] and *S. saprophyticus* [*n*=9; 66.7% (*n*=6) from mushrooms and 33.3% (*n*=3) from hands]. Six isolates that were characterised as multidrug resistant were isolated from hands of handlers. Most (83.9%; *n*=47) of the 56 isolates were resistant to penicillin [53.2% (*n*=25) from mushrooms and 46.8% (*n*=22) from hands] and 14.3% (*n*=8) were resistant to cephalosporin classes [25% (*n*=2) from mushrooms and 75% (*n*=6) from hands], both of which are used to treat staphylococcal infections. Antibiotic resistance genes *blaZ* [25.0% (*n*=14) of all isolates of which 71.4% (*n*=10) were from hands and 28.57% (*n*=4) from mushrooms], *tetL* and *tetK* [both 1.8% (*n*=1) from hands], *mecA* [5.4% (*n*=3) from hands] and *ermA* [1.8% (*n*=1) from mushrooms] were detected from the 56 isolates. Only two (25.0%) of the eight methicillin-resistant staphylococci harboured the *mecA* gene, while only 11 (23%) of the 47 penicillin-resistant isolates harboured the *blaZ* gene [36.4% (*n*=4) from mushrooms and 63.6% (*n*=7) from hands]. Our results demonstrate that food handlers and harvested and packed ready-to-eat mushrooms could be a source of diverse *Staphylococcus* spp. that exhibit antimicrobial resistance. Clinically relevant *S. aureus* was only detected on one handler's hand; however, the isolate was not multidrug resistant. The presence of diverse *Staphylococcus* spp. on mushrooms and the hands of handlers is a potential public health concern due to their potential to cause opportunistic infections.

Significance:
- This study is the first to describe the antibiotic resistance profiles and antibiotic gene presence of *Staphylococcus* spp. isolated from fresh mushrooms and hands of pickers and packers. Mushrooms and handlers in this study were demonstrated to be possible routes of transmission of *Staphylococcus* spp. that are antibiotic resistant and which harbour antibiotic resistance genes, presenting a possible public health hazard.

Introduction

Staphylococcus spp. are ubiquitous and transient organisms. *Staphylococcus aureus* is known to be a natural coloniser of human skin, and between 10% and 20% of adults' skin is persistently colonised by *S. aureus,* while between 30% and 50% of healthy people's skin is colonised by *S. aureus*.[1,2] *Staphylococcus aureus* specifically has been recorded to cause invasive infections or toxin-mediated diseases, including endocarditis, bacteraemia, metastatic infections and toxic shock syndrome.[3] Traditionally, much attention has been paid to *S. aureus* as an organism causing infection; however, coagulase-negative *Staphylococcus* spp. have more recently been shown to also be pathogenic.[4]

Treatment of staphylococcal infections with antibiotics has become common practice in the medical field and, subsequently, higher levels of resistance have been recorded.[2,5] The spread of antimicrobial-resistant staphylococci represents a hazard to human and veterinary health[6] because staphylococci have the ability to transfer antibiotic resistance genes (ARGs) to other pathogenic organisms[2,5]. Previous studies have also shown that staphylococci can be important reservoirs of ARGs in ready-to-eat food.[7,8] Moreover, the exchange of genetic material, such as mobile elements, has reportedly been associated with food-processing environments.[9]

Multidrug-resistant (MDR) staphylococci add to the public health concerns with respect to staphylococcal infections. For example, it has been associated with an increased severity of infections as well as a growing number of people being hospitalised due to such infections.[10] In the United States of America, 60% of infectious disease specialists reported that untreatable bacterial infections had been observed, highlighting the impact on human health.[11] The prevalence of MDR organisms in environmental samples has further heightened the concern of staphylococcal infections for the World Health Organization[10] due to the potential horizontal transfer of ARGs, genetic mutation and recombination. Food associated with bacteria harbouring such ARGs is a major concern and represents possible reservoirs for the spread of these ARGs.[6]

Food commodities that are handled extensively and do not go through a decontamination step might therefore harbour antimicrobial-resistant microorganisms and thus pose a human and environmental health threat. Food products

harbouring antimicrobial-resistant *Escherichia coli*, *Campylobacter* spp., *Salmonella* spp., *Clostridium* spp. and *Listeria monocytogenes* have previously been documented and there has now been an increased interest in the role of *Staphylococcus* spp. isolated from food.[2,6] Therefore, the aim of this study was to determine the diversity of *Staphylococcus* spp. as well as the antibiotic resistance (AR) profiles and the presence of ARGs in isolates obtained from mushrooms and mushroom handlers. Mushrooms that are handled extensively by pickers and packers represent a potential risk if hygiene principles are not observed. This study is the first of its kind to investigate the diversity and AR profiles of *Staphylococcus* spp. on mushrooms and handlers' hands.

Materials and methods

A total of 432 white button mushroom samples were collected as outlined in Rossouw and Korsten[12]. Sampling sites included two large-scale commercial mushroom farms located in Gauteng Province, South Africa, which follow similar production practices, operating under Global-G.A.P. Integrated Farm Assurance Standards V5.1. Mushrooms that were void of defects and at the ready-to-harvest stage were randomly sampled based on uniformity of size, shape and maturity. Mushrooms were aseptically harvested by researchers.[12] Packed mushrooms handled by pickers and packers prior to punnet sealing were also collected. Sampling of hands was done according to standard hand swab procedures.[13] Hand swabs were collected using the Copan Venturi Transystem (Copan, Italy) from five pickers' and five packers' dominant hands on a weekly basis for a period of 15 weeks, resulting in 150 hand samples. Samples were placed in a cooler box and transported to the laboratory for analysis within 24 h. At the time of the study, ethical clearance for non-invasive swabbing of the hands was not mandatory and was therefore waived by the Ethics Committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria. Commercial and personal permission to conduct non-invasive hand swabbing was granted by the commercial entity as well as by the persons whose hands were sampled. Confidentiality was maintained throughout the process.

A sample (250 g) was aseptically obtained from each mushroom sample and homogenised using a handheld blender (Russell Hobbs, Johannesburg, South Africa). Ten grams of the homogenised sample was added to 90 mL tryptone soy broth (Merck, Johannesburg) in a sterile homogeniser bag, macerated for 5 min and incubated for 24 h at 37 °C. Contents were subsequently plated onto Baird-Parker agar (Merck) and incubated for 24 h at 37 °C.

Hand swabs were placed into 9 mL buffered peptone water (Merck), and incubated for 24 h at 37 °C and plated onto Baird-Parker agar plates, which were incubated for 24 h at 37 °C. Presumptive *Staphylococcus* spp. were selected from the Baird-Parker agar, based on a dark grey to black colony morphology which was surrounded by a clear zone.[14] Isolates were purified on Baird-Parker agar and the purified isolate identity was determined using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry in combination with the Bruker Biotyper software and database.[15]

A total of 56 purified and confirmed *Staphylococcus* spp. isolates were used for further characterisation of AR, using the Kirby–Bauer disk diffusion method.[16] Each isolate was cultured in 9 mL of brain heart infusion broth (Merck) and incubated for 18 h at 37 °C (which resulted in approximately log 8 cfu/mL[17]) and subsequently plated onto Mueller–Hinton agar plates (Merck). The Kirby–Bauer disk diffusion test was employed to determine susceptibility to the antibiotics listed in Table 1. Zone diameters were measured and interpreted according to the Clinical and Laboratory Standards Institute guidelines.[17] Strains resistant to three or more antibiotic classes were defined as MDR.

Table 1: Antibiotics tested using the Kirby–Bauer disk diffusion method

Antibiotic class	Antibiotic	Concentration
Cephalosporin	Cefoxitin	30 μg
Phenicol	Chloramphenicol	30 μg
Quinolone	Ciprofloxacin	5 μg
Lincomycin	Clindamycin	2 μg
Macrolide	Erythromycin	15 μg
Aminoglycoside	Gentamicin	10 μg
Penicillin	Oxacillin	1 μg
	Penicillin	10 μg
Rifamycin	Rifampicin	5 μg
Tetracycline	Oxytetracycline	30 μg
Glycopeptide	Teicoplanin	30 μg
	Vancomycin	30 μg

Table 2: Summary of antibiotic resistance genes, associated primers and cycling conditions used for screening purposes

Resistance	Gene		Primers	Amplicon (annealing temperature)	Primer concentration	Reference	Control
Penicillin	*blaZ*	**Forward** **Reverse**	**blaZF** (5'-ACT TCA ACA CCT GCT GCT TTC-3') **blaZR** (5'-TGA CCA CTT TTA TCA GCA ACC-3')	173 bp (55 °C)	1.0 μM	28	*Staphylococcus aureus* ATCC43300
Methicillin/ Oxacillin	mecA	**Forward** **Reverse**	**mecA1** (5'-GGG ATC ATA GCG TCA TTA TTC-3') **mecA2** (5'-AAC GAT TGT GAC ACG ATA GCC-3')	527 bp (50 °C)	0.2 μM	20	*Staphylococcus aureus* ATCC43300
Tetracycline	*tetM*	**Forward** **Reverse**	**tetMF** (5'-AGT GGA GCG ATT ACA GAA-3') **tetMR** (5'-CAT ATG TCC TGG CGT GTC TA-3')	158 bp (48 °C)	0.2 μM	29	None
Tetracycline	*tetK*	**Forward** **Reverse**	**tetKF** (5'-GTA GCG ACA ATA GGT AAT AGT-3') **tetKR** (5'-GTA GTG ACA ATA AAC CTC CTA-3')	460 bp (55 °C)	0.2 μM	29	None
Tetracycline	*tetL*	**Forward** **Reverse**	**tetLF** (5'-TGG TGG AAT GAT AGC CCA TT-3') **tetLR** (5'-CAG GAA TGA CAG CAC GCT AA-3')	229 bp (51 °C)	0.2 μM	30	None
Erythromycin	*ermA*	**Forward** **Reverse**	**ermA1** (5'-AAG CGG TAA AAC CCC TCT GAG-3') **ermA2** (5'-TCA AAG CCT GTC GGA ATT GG-3')	442 bp (52 °C)	0.4 μM	31	None
Erythromycin	*ermC*	**Forward** **Reverse**	**ermC1** (5'-ATC TTT GAA ATC GGC TCA GG-3') **ermC2** (5'-CAA ACC CGT ATT CCA CGA TT-3')	295 bp (50 °C)	0.4 μM	31	None
Erythromycin	*ermB*	**Forward** **Reverse**	**ermBF** (5'-TGG TAT TCC AAA TGC GTA ATG-3') **ermBR** (5'-CTG TGG TAT GGC GGG TAA GT-3')	745 bp (55 °C)	0.4 μM	30	None
Cefoxitin	*ampC*	**Forward** **Reverse**	**ampCF** (5'-GTG ACC AGA TAT GGC CAC A-3') **ampCR** (5'-TTA CTG TAG CGC CTC GAG GA-3')	822 bp (59 °C)	0.6 μM	32	*Enterobacter cloacae* NCTC 13406
16S rRNA amplification control		**Forward** **Reverse**	**27F** (5'-GAG TTT GAT CCT GGC TCA G-3') **1492R** (5'-TAC GGY TAC CTT GTT ACG ACT T-3')	1465 bp	0.4 μM	33	N/A

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Genomic DNA (gDNA) was extracted using the Quick-gDNA Miniprep kit (Zymo Research, Irvine, CA, USA), following overnight incubation in tryptone soy broth at 37 °C. The DNA concentration was measured using the broad range kit for Qubit 2.0 fluorometer (Life Technologies, Johannesburg, South Africa). Polymerase chain reaction (PCR) mixtures with a final volume of 25 μL, containing 10–100 μg gDNA, were prepared using Dream Taq PCR Master Mix (1x) (Thermo Scientific, Johannesburg, South Africa). Each reaction included 16S universal primers as amplification controls (Table 2) as well as the specific ARG primer with primer concentration as outlined in Table 2. PCR cycling conditions were as follows: 94 °C for 2 min, followed by 35 cycles of denaturation (30 s at 95 °C), annealing (30 s at primer temperature, Table 1) and extension (60 s at 72 °C), with a final extension at 72 °C for 5 min. PCR reactions were performed on a BioRad T100 thermocycler (BioRad, Johannesburg, South Africa). Amplicons were visualised on a 2% agarose gel stained with Roti®-safe (Carl Roth GmbH & Co, Karlsruhe, Germany) using a molecular imager in conjunction with the Image Lab™ software (BioRad).

Results

Out of 582 samples collected, 56 (9.6%) were positive for *Staphylococcus* spp. All *Staphylococcus* spp. isolates and their AR and ARG profiles are presented in Figure 1 by sample and year collected. Ten *Staphylococcus* species were isolated: *S. aureus, S. epidermidis*, *S. equorum*, *S. haemolyticus, S. hominis, S. saprophyticus, S. sciuri, S. succinus, S. warneri* and *S. xylosus* (Figures 1 and 2). *Staphylococcus aureus* was only isolated from one packer's hand sample and no mushroom sample yielded *S. aureus*. *Staphylococcus epidermidis* was detected from one picker's and one packer's hand, and both isolates were found to be MDR. A total of 12 *S. equorum* isolates were detected and were isolated mainly from hands (91.67%; *n*=11/12) of pickers (41.67%; *n*=5/12) and packers (50%; *n*=6/12), with only 8.33% (*n*=1/12) isolated from a packed mushroom sample. Two MDR *S. equorum* isolates from pickers' hands, isolated from the same farm and same mushroom picking room (data not shown), shared a phenotypic MDR antibiotic profile but the ARGs were different. Only one MDR *S. haemolyticus* was isolated, from a packer's hand. One packer's hand and one packed mushroom sample were contaminated with *S. hominis*, with the one on the packed mushrooms found to be MDR. Mushrooms sampled before harvest (*n*=6) and three pickers' hands were contaminated with *S. saprophyticus*. Only one *S. sciuri* was isolated from one packed mushroom sample. *Staphylococcus succinus* was isolated from hands (*n*=10) as well as from mushrooms before picking (*n*=4) and after packing (*n*=7), with one found to be methicillin resistant due to the presence of *mecA*. Four of the six *S. succinus* isolates were isolated from pickers in the same growing room during the same sampling period (data not shown). *Staphylococcus xylosus* was only isolated from mushrooms sampled before picking (*n*=4) and after packing (*n*=2) – these isolates were resistant to only the penicillin class of antibiotics. *Staphylococcus warneri* was isolated from a packer's hand.

Six *Staphylococcus* spp. (10.7%; *n*=6/56) were considered MDR, with all isolated from handlers' hands. Expressed resistance to at least one antibiotic agent was found in 85.7% [*n*=48; 52.1% (*n*=25) from mushrooms and 47.9% (*n*=23) from hands] of all *Staphylococcus* spp. characterised. A total of 83.9% of the 56 isolates [*n*=47; 53.2% (*n*=25) from mushrooms and 46.8% (*n*=22) from hands] were resistant to the penicillin class. Resistance to both penicillin and oxacillin was observed in 32 of the 56 isolates, of which 46.9% (*n*=15) were from mushrooms and 53.1% (*n*=17) were from hands.

Of the 47 isolates resistant to the penicillin class, 23.4% (*n*=11) harboured the *blaZ* gene and 6.4% (*n*=3) harboured the *blaZ* gene but did not express resistance to penicillin nor oxacillin. Of the 11 that harboured the *blaZ* gene, 36.4% (*n*=4) were from mushrooms and 63.6% (*n*=7) were from hands. Of the 32 isolates resistant to oxacillin, 9.4% (*n*=3) of isolates from hands harboured the *mecA* gene. Three isolates from hands were resistant to oxytetracycline, with one isolate from hands harbouring *tetK* and one isolate from hands harbouring *tetL*. Only one mushroom isolate harboured *ermA*. No *ampC* was detected, even though eight *Staphylococcus* spp. were resistant to cefoxitin. Two of the three isolates from hands harbouring *mecA* were resistant to cefoxitin.

Discussion

The status of antibiotic-resistant microorganisms in the agricultural environment is becoming a major concern in the global food industry and in the public health sector.[5] The prevalence and status of staphylococci in animal products like meat and cheese have been well established.[5,8,14,18-20] However, there is limited information on the prevalence of AR and ARG in *Staphylococcus* spp. isolated from fresh produce. This study, the first of its kind as far as we could determine, investigated the diversity and resistance of *Staphylococcus* spp. isolated from white button mushrooms and mushroom handlers.

Of the ten *Staphylococcus* species identified, seven were found to be associated with raw mushrooms – these were *S. epidermidis, S. equorum, S. hominis, S. saprophyticus, S. sciuri, S. succinus* and *S. xylosus*. Both *S. aureus* and *S. epidermidis* are associated with the human skin microflora; these as well as other staphylococci pose an important health concern, in addition to exhibiting AR. The presence of these species associated mainly with the hands of workers indicates the need for improved personal hygiene implementation, training and enforcement.[2] These isolates can contribute to illness of both the handlers as well as susceptible consumers.

In this study, *S. saprophyticus*, *S. succinus* and *S. xylosus* were mainly detected from mushrooms. A previous study determined the presence of *S. xylosus, S. epidermidis* and *S. saprophyticus* in ready-to-eat products from animal origin. Furthermore, these species are also commonly associated with farm animals.[8] Mushrooms are cultivated on compost composed of hay, chicken manure, leachate and agricultural lime, which are stored on farms for months in bulk, prior to composting. Antibiotic-resistant bacteria have been found to be present in the excreta of broiler chickens.[21] Graham et al.[21] concluded that typical storage of chicken manure was not sufficient to eliminate the antibiotic-resistant *Staphylococcus* spp. Compost used for the production of mushrooms, which includes chicken manure, is pasteurised at between 60 °C and 75 °C for 13 days. However, Fontes et al.[19] found that staphylococci were able to survive high temperature pasteurisation. Therefore, the presence of these species is not necessarily eliminated from mushroom compost during the pasteurisation process. In addition, previous research has demonstrated that the use of antibiotics in the chicken rearing industry has been linked to the presence of antibiotic-resistant organisms present on farm workers as well as the growing environment.[23] Moreover, flies have previously been reported to increase the human exposure to antibiotic-resistant bacteria.[23] The presence of *Staphylococcus* spp. could be due to survival of organisms during storage and pasteurisation because of the organism's innate ability to survive such conditions as well as the possibility of post-pasteurisation contamination. All these factors might lead to the establishment and spread of *Staphylococcus* species, and antibiotic-resistant and MDR staphylococci in the environment and food system.

There has been an increase in the number of antibiotic-resistant organisms associated with humans[2], their direct environments[20,22,24] and their food[2,8,14,18,19,25]. Transmission of AR in bacteria is further aided by the ability of food to be a vehicle.[8] In the current study, only 10% of all staphylococci isolates were considered MDR. Benjelloun Touimi et al.[2] found that 100% of *Staphylococcus* isolates from vegetable and food handlers were MDR. Moreover, in ready-to-eat food, 94.12%[25], 89.81%[22] and 32.8%[8] of *Staphylococcus* isolates investigated were found to be MDR, reaffirming that food can be a vehicle for the spread of MDR *Staphylococcus* spp.

Penicillin, oxacillin and methicillin are the first line of defence against clinical staphylococci infections. In this study, 83.9% of isolates were resistant to the penicillin class, with 14 isolates harbouring *blaZ*, the gene that encodes *β*-lactamase and confers resistance to penicillin. Similarly, 94% of *Staphylococcus* spp. isolated from chicken and beef[14], 78.5% from soft cheeses[19] and 67.4% from cow mastitis[20] were resistant to penicillin. Moreover, Benjelloun Touimi et al.[2] found that all *Staphylococcus* spp. isolates from ready-to-eat foods were resistant to penicillin and oxacillin. Klimiene et al.[20], however, found a 66% presence of the *blaZ* gene in staphylococci assessed, compared with our study's 25% *blaZ* gene prevalence.
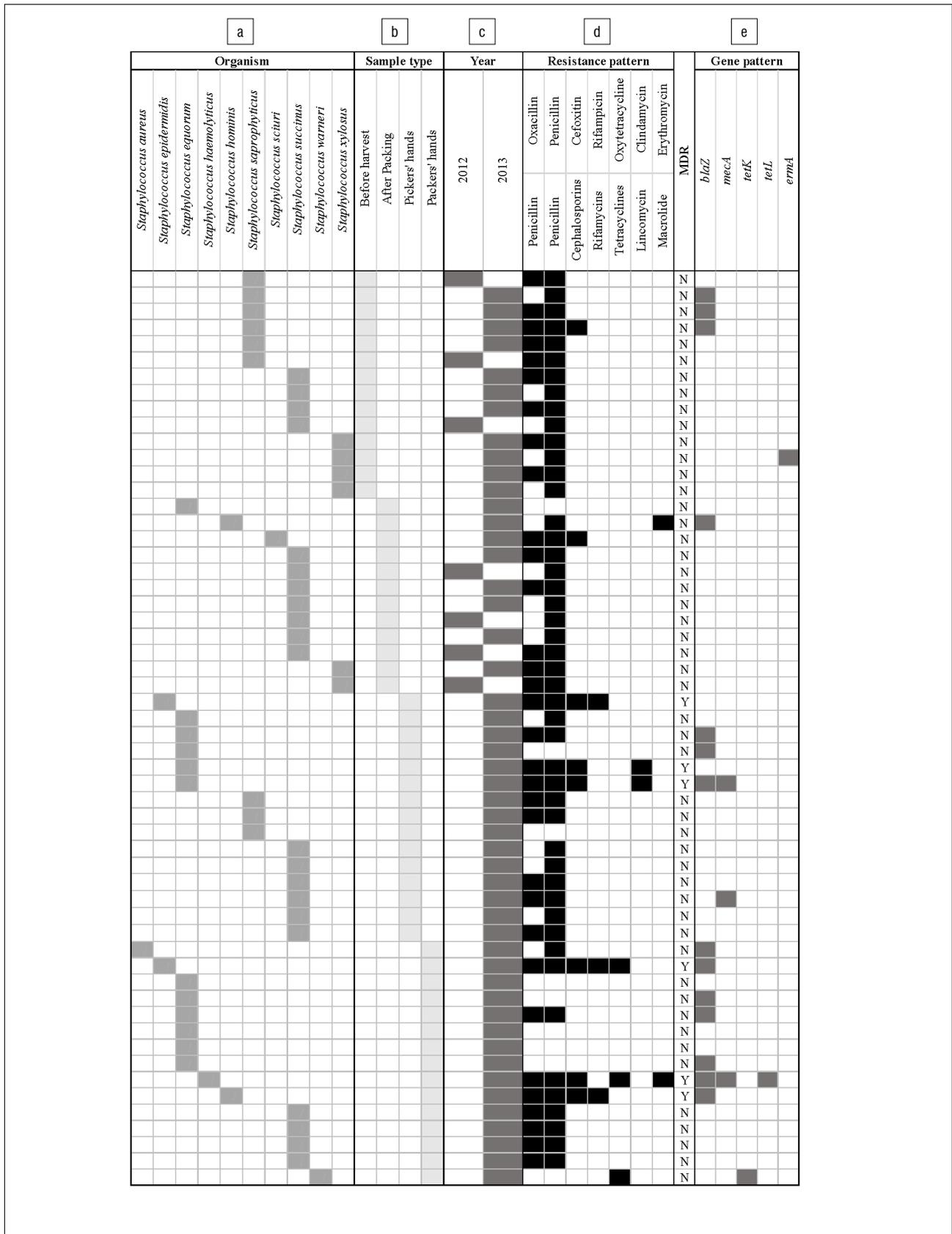
Figure 1: *Staphylococcus* species, phenotypic antibiotic susceptibility profile and antibiotic resistance gene profiles. Each line represents one isolate. (a) Shading indicates to which species the isolate belongs. (b) Shading indicates the isolation source. (c) Shading indicates the year of collection. (d) Shading indicates the resistance to an antibiotic. Only antibiotics for which resistance was determined are included and only antibiotic resistance genes present are listed. (e) Shading indicates the presence of a specified gene.
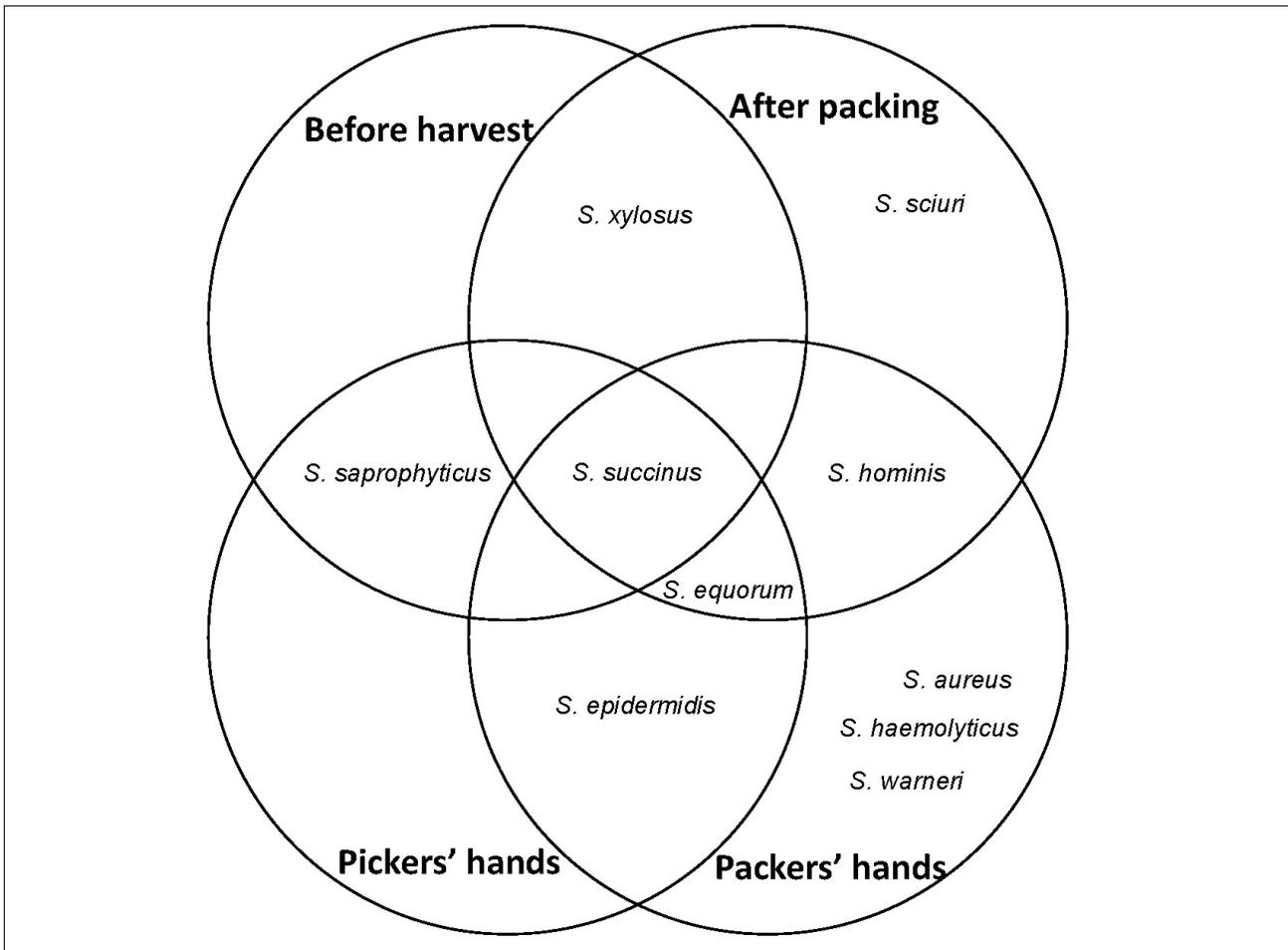
Figure 2: *Staphylococcus* species diversity indicating unique and shared species isolated from ready-to-eat mushrooms at harvest and mushrooms after packing as well as from the hands of pickers and packers.

The resistance of strains to cefoxitin is an indication of methicillin resistance in *Staphylococcus* phenotypes which are resistant to all β-lactam antibiotics, including penicillin and isoxazolyl penicillins.[8] The *mecA* gene confers resistance to methicillin (oxacillin and /or cefoxitin) and its potential transfer between organisms represents a public health concern.[2] In this study, 14.3% ($n=8/56$) of isolates were resistant to cefoxitin, of which 25.0% ($n=2/8$) were found on mushrooms and 75.0% ($n=6/8$) were found on hands; however, only two of the cefoxitin-resistant isolates harboured the *mecA* gene. Vyletelova et al.[26VLKOVA H., MANGA I. (2011] found that not all isolates (174/200) that showed resistance to cefoxitin harboured *mecA*. Chajęcka-Wierzchowska et al.[8] found that all but one isolate exhibiting cefoxitin resistance harboured *mecA*, which is part of the staphylococcal cassette chromosome *mec* (SCCmec) on the bacterial chromosome. All isolates in the current study that were resistant to cefoxitin were also resistant to oxacillin and penicillin. The co-occurrence of resistance to methicillin, oxacillin and penicillin has been described previously.[26] In addition, Chajęcka-Wierzchowska et al.[8] reported that isolates with resistance to methicillin, oxacillin and penicillin were also resistant to rifampicin, clindamycin and tetracycline. In this study, 33.3% of the eight methicillin-resistant staphylococci were also resistant to rifampicin, 25% to tetracycline and clindamycin and 25% to erythromycin.

Two mechanisms of tetracycline resistance have been identified in *Staphylococcus* spp. and are mediated by *tet* genes: plasmid-mediated *tetK* and *tetL* encoding efflux and *tetM* encoding ribosomal protection mediated determinants.[27] In the current study, two isolates harbouring *tetL* and *tetK* were found to be resistant to oxytetracycline; in addition, one isolate was found to be resistant to oxytetracycline without the presence of any of the *tet* genes tested for. Previous studies have found discrepancies between phenotypic expression and the presence of resistance genes.[27] In comparison, AR to tetracycline was seen in 14.7% of staphylococci isolates from soft cheese[19], 18.9% of staphylococci isolates from cow mastitis[20] and 34.5% of isolates from food of animal origin, of which all isolates resistant harboured at least one *tet* gene[8]. Osman et al.[14] found 68% AR to tetracycline from raw beef and chicken meat in Egypt, which was considerably higher than this and other studies. A conjugative transposon (Tn6079) is responsible for the spread of *tetL*[27], whereas *tetM* can be transposon-located or chromosomal[27]. Therefore, the presence of *tetL* and *tetM* can indicate the potential for horizontal gene transfer between organisms.

Conclusion

In conclusion, a diverse number of *Staphylococcus* spp. were associated with mushrooms and mushroom handlers' hands. Antibiotic resistance of these mushroom- and hand-associated *Staphylococcus* spp. demonstrates a public health threat due to the potential of antibiotic gene transfer to medically important *Staphylococcus* spp. Moreover, opportunistic infection that might result due to an AR *Staphylococcus* spp. could lead to an infection that is difficult to treat. The presence of AR organisms adds to the general concern around the reservoir of AR on food products and food handlers' hands. Future research should determine the source of *Staphylococcus* spp. in production and on the product at the market end, in order to determine the specific risk to the final consumer. In addition, future research should also focus on ARG transfer and mechanisms in *Staphylococcus* species within the agricultural environment.

Acknowledgements

Competing interests

We have no competing interests to declare.

Authors' contributions

S.D.: Conceptualisation, research design and interpretation of data (conceptualisation), laboratory work, data collection and analysis (methodology and data collection), framework, final revision of manuscript, editing for intellectual content and submission (drafting or critically revising the manuscript), project management. W.R.: Research design (conceptualisation), field work, sampling, laboratory work, data collection and analysis (methodology and data collection), framework and writing of manuscript (drafting or critically revising the manuscript), project management. G.V.R.: Conceptualisation, research design and interpretation of data (conceptualisation), laboratory work, data collection and analysis (methodology and data collection), framework, final revision of manuscript, editing for intellectual content (critically revising the manuscript). E.d.P: Conceptualisation, research design and interpretation of data (conceptualisation), data analysis (methodology and data collection), framework, final revision of manuscript, editing for intellectual content and submission (drafting or critically revising the manuscript). L.K.: Research design (conceptualisation), data analysis (methodology and data collection), framework, final revision of manuscript, editing for intellectual content and submission (drafting or critically revising the manuscript), student supervision, project leadership, funding acquisition.

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