PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	An observational cross-sectional study of nasal staphylococcal species of medical students of diverse geographical origin, prior to healthcare exposure. Prevalence of SCCmec, fusC, fusB and the arginine catabolite mobile element (ACME) in the absence of selective antibiotic pressure.
AUTHORS	Budri, Paulo; Shore, Anna; Coleman, David; Kinnevey, Peter; Humpreys, Hilary; Fitzgerald-Hughes, Deirdre

VERSION 1 – REVIEW

REVIEWER	Noriko Urushibara, PhD Sapporo Medical University School of Medicine, Japan
REVIEW RETURNED	21-Nov-2017

GENERAL COMMENTS	This paper genetically characterized the co-located nasal S. aureus and coagulase-negative staphylococci (CoNS) recovered from healthy medical students in their pre-clinical year. A14-month prospective cross-section study was conducted on medical students from geographically diverse origins in a single hospital in Ireland. Nasal swabs collected from 444 students, and analyzed for antimicrobial resistance and virulence-associated genes. The authors focus on S. aureus and CoNS co-carried on the same swabs. The rates of co-carriage of antibiotic resistance genes in paired S. aureus/CoNS from the same individual were low at <1%. The targeted resistance genes were more prevalent among CoNS than in S. aureus. These results suggest a low rate of transfer of antibiotic resistance between colonizing staphylococcal species at least in the absence of antibiotic pressure. In addition, high prevalence of resistance gene in CoNS supports the common recognition that CoNS acts as a community reservoir of these genes. The study design and the employed method seem sufficient. Obtained data will be informative. Therefore, I think that the manuscript is suitable for JMBopen. Though the authors did not attempt to follow-up of this cohort, it would be of great interest how the carriage of antimicrobial resistance genes will change during clinical training period.

	I have provided some comments and suggestions below that, I think, would improve impact and clarity of the study. Introduction Line 77: Change "SCCmecIV" to "SCCmec IV". Please enter a space between SCCmec and IV. Line 87: Please spell out "CC 1" (clonal complex 1). Methods Lines 122-125: How were the Staphylococcal isolates selected from the SaSelect agar plates? What were the selection criteria? Line 140: Change "carriage of fusC, fusB and tst1" to "carriage of fusidic acid resistance genes fusC, fusB, and toxic shock syndrome toxin gene tst1"; The biological function of a gene or a gene product should be described the first time it appeared in the main text, not at its second use (Line 146). Results Lines 157-162: S. aureus was detected in 137/444. Did the all S. aureus-positive students possess CoNS? Lines 157-166: This paragraph was described only in the manuscript. It makes difficult to follow. May I suggest creating a new additional table describing these data? Line 178: Please provide the readers with more information on the genetic features of CC1-MRSA-V-SCCfus: e.g. characteristic hybridization patterns on the microarray Genotyping Kit 2.0. Lines 184-186: "In addition to the three CC1-MRSA-V isolates that carried SCCfus, the fusidic acid resistance genes fusC and fusB were identified in 28/128 (21.8%) and 2/128 (1.5%) of methicillin-susceptible S. aureus (MSSA) isolates, respectively." The fusC gene was, then, detected in 31/137 S. aureus isolates (3 + 28 = 31)? Table 1 indicates that 30 S. aureus harbor fusC gene. Please clarify. Line 188: Please spell out "ccr" (cassette chromosome recombinase). Line 189: Change "ccrA1 ccrB-1" to either of the followings, "ccrA-1 and ccrB-1" or "ccrA1 and ccrB1". ccr gene name should be italicized including "A1" and "B1". The same shall apply hereafter. Alternatively, it can be abbreviated after the first appearance, e.g. ccrA1/B1. Lines 190-194: Among MR-CoNS, five S. epidermidis carried fusC, and nine CoNS isolates harboured fusB. Among methicillin susceptible CoNS isolates, fusC and fusB genes were identified in 20 and 18 isolates, respectively. Accordingly, fusC and fusB genes were detected in 25 and 27 CoNS isolates among the 137 CoNS from the participants with co-colonized with S. aureus. Table 1 indicates that 20 and 18 isolates were positive for fusC and fusB, respectively. Please clarify. Lines 221-222: Rephrase "The staphylococcal isolates were

	negative for all other antibiotic resistance genes detected by the microarray." Suggestion: "The staphylococcal isolates were negative for all other antibiotic resistance genes spotted on the microarray." The same shall apply in the description in lines 233-234. Discussion Line 271: Change "CC1-MRSA-V+SCCfusC" to "CC1-MRSA-V-SCCfusC". Lines 271-272: What does "WA" stand for? Western Australia? Lines 290-291: Does "this SCCmec element" mean the "SCCmec IV"? Lines 295-296: "However, we found a prevalence of 21.9% of fusC/fusB genes among healthy carriers." It would be useful to clarify how many isolates among all the CoNS (n=386) carried fusC/fusB here. In the results section, prevalence of fusC/fusB was described only among the 137 S. aureus/CoNS pairs. Line 309: It is not clear what the authors mean by "a single mobile element"? Does this relate to SCCfus, SCCmec, or a composite element including the two SCCs? Lines 333-334: A pair of S. aureus/CoNS pair from one student carried the same type of SCCmec, SCCmec IV (described in lines 182-183). In addition, one participant had nasal co-carriage of fusC-positive S. aureus and CoNS (described in lines 195-196). Do the authors conclude that the resistance gene (mecA or fusC) had been transferred form the CoNS to the S. aureus in each participant? Table Table 1 Line 377: Please rephrase the table heading to something like: "Resistance/virulence genes detected among 137 co-located nasal S. aureus/CoNS pairs". Line 378: Please rephrase "associated with SCC element, (ccrA1 and ccrB-) in 13/137 S. aureus". Figure Legends Figure 2 Line 4: Change "grey bars" to "dark grey bars". Change "white bars" to "light grey bars". References Journal names should be abbreviated according to Index Medicus journal abbreviations. In addition, the journal title is missing in some references (e.g. Ref 5).

REVIEWER	Bernhard Krismer University of Tuebingen Germany
REVIEW RETURNED	15-Dec-2017

GENERAL COMMENTS	Manuscript bmjopen-2017-020391 In the presented manuscript, the authors investigate the (co-)carriage of Staphylococcus aureus and coagulase-negative Staphylococcus species (CoNS) in healthy undergraduate students

	during their preclinical year. They correlate the presence of various resistance genes, including staphylococcal cassette chromosome mec (SCCmec) and fusB/fusC, and virulence factors in strain pairs isolated from the same carriers. Although the study presents some interesting findings, like a very low co-occurrence of methicillin resistant S. aureus and CoNS in the same carriers, the manuscript needs some revisions to be acceptable. Species names should not be abbreviated at their first mention. Therefore, the full names Staphylococcus aureus and Staphylococcus epidermidis should be written also in the abstract, not only in the introduction. Additionally, the terms CoNS and MRSA are not explained in the abstract. The authors describe the isolation of S. aureus and S. epidermidis as dominant species and a very low percentage of Staphylococcus haemolyticus and Staphylococcus saprophyticus. Except for S. aureus and S. epidermidis this is an atypical isolation result, since most literature dealing with isolation frequencies show S. hominis, S. capitis, S. warneri and S. lugdunensis as main colonisers (beside the two dominant species S. aureus and S. epidermidis). It is very likely that the method of sample preparation distorted the original species distribution since mannitol salt broth and SaSelect agar clearly select for S. aureus and other pathogenic species. Slow growing species and mannitol non-fermenters like the aforementioned S. lugdunensis and S. hominis are easily overgrown by S. aureus. This should be mentioned by the authors as a putative reason for the absence of otherwise abundant species (also in the discussion). Page 5, line 120: “relevant”; line 125: Bruker Page 7, line 158: this 6.5% MRSA leads to confusion, since in the abstract the value of 2 % was used. Either calculate the carriage rate for the number of participants or the S. aureus carriers, but don’t change the calculation between abstract and results. Line 159-160: the frequency of isolation of these species should be compared with other publications since they are rather uncommon in the nares. Page 9, line 210 ff.: CoNS numbers are given as 137 like the numbers of S. aureus. This indicates that all S. aureus carriers were also colonised by one CoNS species. This should be highlighted in the manuscript (or otherwise explained). Page 11, lines 264 ff.: the authors underline that the clonal complexes CC30, CC88 and CC8 were the most prevalent clones identified among nasal S. aureus and are typically associated with SCCmec carriage. Then, they indicate that their MRSA isolates exclusively belong to CC59 and CC1. It should be more pronounced that – in contrast to the expectation - their CC30, CC88 and CC8 isolates are MSSA. Page 12, line 302: explain SSTI Pages 15 and 26: these are different Figure legends for the same Figure 1! Also the description for the grey bars differ! In this Figure not only the absolute numbers of S. aureus carriers should be indicated but also the percentage (like in the results).

	(Figure 1. The geographical origin of 444 medical students from whom a nasal swab was collected (dark grey bars) and the proportion of participants with nasal co-carriage of S. aureus and CoNS (light grey bars). (Figure 1. Geographical origin of medical students recruited. The geographical areas of origin of 444 medical students recruited to the study are shown (dark grey bars). Of those recruited, 137 were confirmed nasal S. aureus positive. The proportion of recruited students from each geographical origin with nasal S. aureus carriage are also shown (light grey bars). Page 27, Figure 2: The Figure legend says "Figure 1" although it is Fig. 2. The legend indicates grey bars, white bars and bold lettering. The Figure shows dark grey, light grey and no bold lettering. Instead, the description (a) is used but not explained. Therefore, completely revise the Figure legend. A previous publication (Methicillin and multidrug resistant coagulase negative staphylococcal nasal carriage in medical students; Baragundi et al.; J Commun Dis. 2012 Dec;44(4):231-7) describes the distribution of MR-CoNS in medical students. This might be considered in the discussion.

VERSION 1 – AUTHOR RESPONSE

Editorial requirements:

- Along with your revised manuscript, please include a copy of the STROBE checklist indicating the page/line numbers of your manuscript where the relevant information can be found (https://strobe-statement.org/index.php?id=strobe-home)
We have completed this document and have uploaded it in the revised submission

R- Please revise the 'Strengths and limitations' section of your manuscript. This section should relate specifically to the methods, and should not include a general summary of, or the results of, the study.
A: The section has been revised as requested and is now focused on the methods and design.

Reviewer 1 comments, responses and text changes
R: Line 77: Change "SCCmecIV" to "SCCmec IV". Please enter a space between SCCmec and IV.
A: This change has been made, line 77

R: Line 87: Please spell out "CC 1" (clonal complex 1).
A: Acronym has now been spelled out, line 87

R: Lines 122-125: How were the Staphylococcal isolates selected from the SaSelect agar plates? What were the selection criteria?
A: Selection was based on colony colour on this chromogenic agar, as outlined in lines 118-121, however we have further clarified this in the text.

R: Line 140: Change “carriage of fusC, fusB and tst1” to “carriage of fusidic acid resistance genes fusC, fusB, and toxic shock syndrome toxin gene tst1”; The biological function of a gene or a gene product should be described the first time it appeared in the main text, not at its second use (Line 146).

A: The genes descriptions have been added/edited as suggested in lines 141-142.

Results

R: Lines 157-162: S. aureus was detected in 137/444. Did the all S. aureus-positive students possess CoNS?

A: Yes, all students positive for S. aureus were positive for CoNS as well. And this is now state in line 161 ‘All students positive for S. aureus also carried S. epidermidis.’

R: Lines 157-166: This paragraph was described only in the manuscript. It makes difficult to follow. May I suggest creating a new additional table describing these data?

A: We have included an additional table to clarify these results. The table is referred to in line 161 and included as Table 1, line 388.

R: Line 178: Please provide the readers with more information on the genetic features of CC1-MRSA-V-SCCfus: e.g. characteristic hybridization patterns on the microarray Genotyping Kit 2.0.

A: We have included in brackets, the genes on the array that distinguish SCCmec types V and VI and SCCfus, lines 180-183

R: Lines 184-186: “In addition to the three CC1-MRSA-V isolates that carried SCCfus, the Fusidic acid resistance genes fusC and fusB were identified in 28/128 (21.8%) and 2/128 (1.5%) of methicillin-susceptible S. aureus (MSSA) isolates, respectively.” The fusC gene was, then, detected in 31/137 S. aureus isolates (3 + 28 = 31)? Table 1 indicates that 30 S. aureus harbour fusC gene. Please clarify.

A: This typing error has been corrected in the table.

R: Line 188: Please spell out “ccr” (cassette chromosome recombinase).

A: The spell out was added in line 190.

R: Line 189: Change “ccrA1 ccrB-1” to either of the followings, “ccrA-1 and ccrB-1” or “ccrA1 and ccrB1”. ccr gene name should be italicized including “A1” and “B1”. The same shall apply hereafter. Alternatively, it can be abbreviated after the first appearance, e.g. ccrA1/B1.

A: The typing errors have been corrected here and throughout the text

R: Lines 190-194: Among MR-CoNS, five S. epidermidis carried fusC, and nine CoNS isolates harboured fusB. Among methicillin susceptible CoNS isolates, fusC and fusB genes were identified in

20 and 18 isolates, respectively. Accordingly, fusC and fusB genes were detected in 25 and 27 CoNS isolates among the 137 CoNS from the participants with co-colonized with S. aureus. Table 1 indicates that 20 and 18 isolates were positive for fusC and fusB, respectively.
Please clarify.

A: The figures have been corrected and are now consistent in the table and text

R: Lines 221-222: Rephrase "The staphylococcal isolates were negative for all other antibiotic resistance genes detected by the microarray." Suggestion: "The staphylococcal isolates were negative for all other antibiotic resistance genes spotted on the microarray." The same shall apply in the description in lines 233-234.

A: The text has been edited as suggested, lines 226-227, 238-239

Discussion

R: Line 271: Change "CC1-MRSA-V+SCCfusC" to "CC1-MRSA-V-SCCfusC".
Lines 271-272: What does "WA" stand for? Western Australia?
Lines 290-291: Does "this SCCmec element" mean the "SCCmec IV"?

A: These three edits have been made as suggested

R: Lines 295-296: "However, we found a prevalence of 21.9% of fusC/fusB genes among healthy carriers." It would be useful to clarify how many isolates among all the CoNS (n=386) carried fusC/fusB here. In the results section, prevalence of fusC/fusB was described only among the 137 S. aureus/CoNS pairs.

A: We limited our investigation of antibiotic resistance/virulence gene to the 137 x 2 paired isolates (S. aureus and CoNS) recovered together from the same nare. Therefore we did not record the prevalence of fusC/fusB in all the S.epidermidis recovered. We had stated this as a limitation but have further clarified this as follow:

Line 332-334 '…….. and therefore prevalence rates for genes among CoNS do not reflect the entire cohort.'

R: Line 309: It is not clear what the authors mean by "a single mobile element"? Does this relate to SCCfus, SCCmec, or a composite element including the two SCCs?

A: Yes, this refers to a composite element, SCCmec V+SCCfus. We have included this in line 313 and have replaced this term with "a single transfer event" (line 318)

R: Lines 333-334: A pair of S. aureus/CoNS pair from one student carried the same type of SCCmec, SCCmec IV (described in lines 182-183). In addition, one participant had nasal co-carriage of fusC-positive S. aureus and CoNS (described in lines 195-196). Do the authors conclude that the

resistance gene (mecA or fusC) had been transferred form the CoNS to the S. aureus in each participant?

A: While this is a possibility, we have been cautious to avoid this conclusion. We do not have direct evidence of such a transfer and it would be difficult to show this in-vivo. It could be argued that these entities (resistant S. aureus and CoNS) could have been acquired separately and coincidently but not necessarily through gene transfer from a resistant CoNS to S. aureus in the nare.
Table

Table 1

R: Line 377: Please rephrase the table heading to something like: “Resistance/virulence genes detected among 137 co-located nasal S. aureus/CoNS pairs”.

A: the table (Table 2 in the revised version) heading was revised as suggested

Line 378: Please rephrase “associated with SCC element, (ccrA1 and ccrB-) in 13/137 S. aureus”.

A:text revised as suggested

Figure Legends
Figure 2

Line 4: Change “grey bars” to “dark grey bars”. Change “white bars” to “light grey bars”.

A: The typing error was corrected in the revised legend

References

Journal names should be abbreviated according to Index Medicus journal abbreviations. In addition, the journal title is missing in some references (e.g. Ref 5).

A: The references were revised and corrected.

Reviewer 1 comments, responses and text changes

R. Species names should not be abbreviated at their first mention. Therefore, the full names Staphylococcus aureus and Staphylococcus epidermidis should be written also in the abstract, not only in the introduction. Additionally, the terms CoNS and MRSA are not explained in the abstract.
A: Full name of species and terms are now spelled out in full in the abstract.

R. The authors describe the isolation of S. aureus and S. epidermidis as dominant species and a very low percentage of Staphylococcus haemolyticus and Staphylococcus saprophyticus. Except for S. aureus and S. epidermidis this is an atypical isolation result, since most literature dealing with isolation frequencies show S. hominis, S. capitis, S. warneri and S. lugdunensis as main colonisers (beside the two dominant species S. aureus and S. epidermidis). It is very likely that the method of sample preparation distorted the original species distribution since mannitol salt broth and SaSelect

agar clearly select for S. aureus and other pathogenic species. Slow growing species and mannitol non-fermenters like the aforementioned S. lugdunensis and S. hominis are easily overgrown by S. aureus. This should be mentioned by the authors as a putative reason for the absence of otherwise abundant species (also in the discussion).
A: We agree that our methods possibly selected for S. aureus and S. epidermidis. We have clarified: Line 116-117: '…to recover S. aureus (including MRSA) and pathogenic CoNS species' Lines 330-334. 'Some nasally abundant CoNS species, for example Staphylococcus lugdunensis and Staphylococcus hominis were under-represented as the enrichment method favoured pathogenic staphylococci such as S. aureus and S. epidermidis.'

R: Page 5, line 120: "relevant"; line 125: Bruker
A: The typing errors have been corrected, lines 120 and 125.

R: Page 7, line 158: this 6.5% MRSA leads to confusion, since in the abstract the value of 2 % was used. Either calculate the carriage rate for the number of participants or the S. aureus carriers, but don't change the calculation between abstract and results.
A: We have clarified these figures and percentages and kept them consistent at 6.6 % (9/137) in abstract and results, Lines 38 and 159.

R: Line 159-160: the frequency of isolation of these species should be compared with other publications since they are rather uncommon in the nares. (what is referred to here,
A: these are indeed, reported to be uncommon colonisers of the nares at least in a community setting. In any case, our methods preferentially favour the growth of S. aureus and S. epidermidis (as clarified in response to comment of reviewer 1) and therefore this may not be a useful comparison. We have further qualified this in the discussion lines 246-248

R: Page 9, line 210 ff.: CoNS numbers are given as 137 like the numbers of S. aureus. This indicates that all S. aureus carriers were also colonised by one CoNS species. This should be highlighted in the manuscript (or otherwise explained).
A: This is correct and is now stated, Lines 161-162 'All students positive for S. aureus also carried S. epidermidis'

R: Page 11, lines 264 ff.: the authors underline that the clonal complexes CC30, CC88 and CC8 were the most prevalent clones identified among nasal S. aureus and are typically associated with SCCmec carriage. Then, they indicate that their MRSA isolates exclusively belong to CC59 and CC1. It should be more pronounced that – in contrast to the expectation - their CC30, CC88 and CC8 isolates are MSSA.
A: While these CCs are MSSA lineages into which SCCmec can integrate, SCCmec has not integrated in all representatives of these CCs. Therefore, we did not expect all MRSAs to belong to these lineages. Rather we wanted to highlight that there is potential for uptake of SCCmec as the lineages present can acquire this element (as opposed to lineages that are not reported to acquire SCCmec). We have clarified this paragraph to reflect this perspective as follows:
Lines 272 - changed 'strain' to 'lineage'
Lines 273-274 'Among medical students, these MSSA isolates may therefore represent a significant pool for the uptake of SCCmec in a clinical setting'
Lines 277-279 'Although CC30, CC88 and CC8 were prevalent among community MSSA isolates in this study, among the relatively few MRSA recovered, none belonged to these CCs.'

R: Page 12, line 302: explain SSTI
A: This acronym has now been spelled out.

R: Pages 15 and 26: these are different Figure legends for the same Figure 1! Also the description for the grey bars differ! In this Figure not only the absolute numbers of S. aureus carriers should be indicated but also the percentage (like in the results).
A: The correct figure legends have now been assigned. The bar colours and format have been corrected so that they are consistent between the legend and the figure.

R: A previous publication (Methicillin and multidrug resistant coagulase negative staphylococcal nasal carriage in medical students; Baragundi et al.; J Commun Dis. 2012 Dec;44(4):231-7) describes the distribution of MR-CoNS in medical students. This might be considered in the discussion.
A: we have added the results of this publication to the discussion. Line 292-293
'One study reported an increasing in carriage rates of MRCoNS from 14% among medical student pre-internship, to 29.28% among interns.'

FORMATTING AMENDMENTS (if any)
Required amendments will be listed here; please include these changes in your revised version:

VERSION 2 – REVIEW

REVIEWER	Noriko Urushibara Department of Hygiene, Sapporo Medical University School of Medicine, Japan
REVIEW RETURNED	22-Jan-2018

GENERAL COMMENTS	I am really satisfied with the authors' corrections. I found a typo in the table note of newly made Table 1, but it can be easily addressed without a need for re-review: Line 409: "MRSE + methicillin resistant S. epidermidis" should be "MRSE = methicillin resistant S. epidermidis"

REVIEWER	Bernhard Krismer University of Tuebingen Germany
REVIEW RETURNED	02-Feb-2018

GENERAL COMMENTS	only minor corrections are suggested: Due to the insertion of the genotypes (lines 183 ff) the first appearance of "ccr" is in line 184. The explanation "cassette chromosome recombinase genes (ccr)" is mentioned only in lines 196/197. on page 17, Table 1: Putting the percentage numbers and the %-sign in the header in parenthesis would significantly improve readability. Lines 397 and 399: change "Staphylococci" and "Staphylococcal" into "Staphylococcus" -The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

VERSION 2 – AUTHOR RESPONSE

Dear Editor,

We would like to thank the reviewers and the editor for their careful review of this manuscript and helpful suggestion for its improvement. We corrected all minors details. Changes to the manuscript are highlighted in yellow in the uploaded revised manuscript. We hope that the manuscript now meets your requirements.

(R1) Line 409: “MRSE + methicillin resistant S. epidermidis” should be “MRSE = methicillin resistant S. epidermidis”

(A) The table note was revised and the mistake was corrected.

(R2) Due to the insertion of the genotypes (lines 183 ff) the first appearance of "ccr" is in line 184. The explanation "cassette chromosome recombinase genes (ccr)" is mentioned only in lines 196/197.

(A) The explanation was corrected and included in the right place.

(R2) on page 17, Table 1: Putting the percentage numbers and the %-sign in the header in parenthesis would significantly improve readability.

(A) The parenthesis' were included in the new version.

(R2) Lines 397 and 399: change "Staphylococci" and "Staphylococcal" into "Staphylococcus"

(A) The change was included in the new version.

Thank you!

Paulo Eduardo Budri.