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ORAL CAVITY COLONIZATION WITH MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA AFTER PREOPERATIVE PROPHYLACTIC USE OF ANTIBIOTICS AS A RISK FACTOR FOR VENTILATOR-ASSOCIATED PNEUMONIA

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SUMMARY

Background: *Although it was previously shown that prolonged prophylactic antibiotic exposure and multiple inadequate antibiotic therapies are independent risk factors for multidrug-resistant ventilator associated pneumonia there were no studies investigating whether pre-operative prophylactic dose of antibiotics changes oral microbiome and increases the risk of ventilator associated pneumonia. The aim of the study was to determine if pre-operative prophylactic dose of antibiotics affects the oral microbiome, increases the colonization with Gram-negative bacteria and subsequent risk of ventilator associated pneumonia.*

Subjects and methods: *Mechanically ventilated adult patients receiving surgical antibiotic prophylaxis were included in the study. The presence of Gram negative microorganisms in the pre-prophylactic and post-prophylactic oral swabs and tracheal aspirates, as well as the occurrence of ventilator associated pneumonia, were analyzed.*

Results: *Number of patients colonized with Gram negative bacteria in post- prophylactic oral swab was significantly higher compared to oral swab taken before prophylactic antibiotic. On the other hand, the number of patients with Gram- negative bacteria in tracheal aspirates remained similar as in post- prophylactic oral swabs. Moreover, we found that presence of Gram- negative bacteria in both pre- and post- prophylactic oral swabs was in the positive correlation with the presence of Gram- negative bacteria in tracheal aspirates.*

Conclusions: *This study showed increased colonization of oral cavity with Gram- negative bacteria after preoperative prophylactic antibiotics. Furthermore, receiving two prophylactic antibiotics from WHO Watch list increased the incidence of Gram-negative bacteria in oral swabs and tracheal aspirates, and the risk of ventilator associated pneumonia development.*

Key words: *ventilator associated pneumonia - oral cavity colonization -multidrug-resistant gram-negative bacteria - antibiotic prophylaxis*

* * * * *

INTRODUCTION

Ventilator associated pneumonia (VAP) is a serious and common complication in ventilated patients in the intensive care unit (ICU) and contributes to mortality significantly (Koulenti et al. 2006, Chastre & Fagon 2002). It is a form of hospital acquired pneumonia developing in mechanically ventilated patients more than 48 h after intubation (Dey & Bairy 2007). VAP predominantly occurs in ICUs and the rate varies from 9-27% in intubated patients, whereas the death rate is 20-70% (Patro et al. 2018, Craven 2000). VAP developing within the first 4 days (early-onset VAP) is usually associated with susceptible bacteria (such as Streptococcus pneumonia or Haemophilus influenzae), while VAP emerging after 4 days (late-onset VAP) and is usually caused by Gram-negative bacteria (such as Acinetobacter baumanni, Pseudomonas aeruginosa, and Klebsiella pneumoniae), which are in 61% cases multidrug-resistant (MDR) (Patro et al. 2018, Dey & Bairy 2007). Unfortunately, nowadays the incidence of multidrug resistant Gram-negative (MDR-GN) pathogens is relatively high in ICU setting (Craven 2000). VAP prolongs the duration of mechanical ventilation and the length of hospital stay thus increasing the costs of health care system. VAP is responsible for 50% of medication cost in ICUs (Pugin at al. 1991).

The oral cavity contains a number of different habitats, thus it provides the appropriate habitat for numerous microorganisms: more than 700 bacterial species may be found in the human oral cavity (oral microflora, microbiota, or microbiome) (Paster et al. 2006, Dewhirst et al.

2010). Imbalance of oral microflora contributes to oral and systemic diseases (Han & Wang 2013).

Although it was previously shown that prolonged prophylactic antibiotic exposure and multiple inadequate antibiotic therapies are independent risk factors for multidrug-resistant VAP (Sands et al. 2017), there were no studies investigating whether pre-operative prophylactic dose of antibiotics changes oral microbiome and how these changes affect the incidence and causative agents of VAP.

The aim of the study was to determine the impact of pre-operative prophylactic dose of antibiotics on oral cavity colonization. Secondly, we investigated the role of the colonization of oral cavity with multidrug-resistant Gram-negative bacteria in the development of VAP and the impact of antibiotic resistance on the mortality of patients and length of hospital stay. Furthermore, we analyzed which WHO group of antibiotics given in the surgical prophylaxis select - negative bacteria and the resistant strains in the oral cavity and whether the resistant phenotype affects the need for antibiotic escalation in patients developing VAP.

SUBJECTS AND METHODS

Subjects

The study included adults (over 18 years), mechanically ventilated patients receiving surgical antibiotic prophylaxis in three intensive care units of the University Hospital Centre Zagreb (cardiac-AKA, general surgical- AIK, and neurosurgical-AIN). Patients' demographic data and outcome, antibiotics used for preoperative prophylaxis, duration of ICU and hospital stay, as well as the number of ventilator hours were recorded. Moreover, the presence of Gram- negative microorganisms in the pre-prophylactic and post-prophylactic oral swabs, and tracheal aspirates, as well as the occurrence of VAP were analyzed. The diagnosis of VAP was based on chest X-ray, elevated C-reactive protein (CRP) levels and polymorphonuclear count. *Written informed consent was obtained from all patients participating in the trial. Study was approved by the Ethical Committee of the University Clinical Hospital Center Zagreb.*

Methods

There were 21 different antibiotics as listed in Table 1, or a combination of two or three of these agents used in perioperative prophylaxis. Patients received prophylaxis with antibiotics commonly used in intensive care units such as cefazolin, cefuroxime, metronidazole, piperacillin in combination with tazobactam or cloxacillin and antibiotic therapy was changed, if necessary, after the antibiogram result. Antibiotic choice on the clinical picture and the type of procedure to be applied to the and whether it was primarily "clean" procedure such as skull or heart surgery or procedures that are at risk of contamination such with intestinal flora such as abdominal surgery.

The first samples of oral swabs and endotracheal aspirates (ETA) were taken immediately before the introduction of antibiotic therapy, and the second swabs on the fifth day after the introduction of antibiotic therapy. The oral swab and ETA were taken by an educated bachelor of nursing. The samples were immediately taken to the laboratory, or if not possible, stored in the refrigerator. The first oral swabs were seeded on blood agar plates according to the routine diagnostic procedure. The second oral swab was seeded on selective medium for Gram-negative bacteria Mac- Conkey agar (Copan, Zagreb). The ETA samples

Table 1. Antibiotics used in perioperative prophylaxis classified accoriding to WHO's AWaRe classification of antibiotics

Group of antibiotics		
Access*	Watch*	Reserve**
amikacin; amoxyicilin/clavulanic acid	cefepime	colistin
benzylpenicillin	ceftazidime	linezolid
cephazolin	ceftriaxone	
clindamycin	cefuroxime	
cloxacilin	ciprofloxacine	
gentamicin	ertapneme	
metronidazole	meropeneme	
sulfametoxazole+trimethoprim	piperacilin/tazobactam	
	rifampicin	
	teicoplanin	
	vancomycin	

* Antibiotics that have activity against a wide range of commonly encountered susceptible pathogens, also showing lower resistance potential than antibiotics in the other groups and are recommended as essential first or second choice empiric treatment options; * Antibiotic classes that have higher resistance potential and/or antibiotics that are at relatively high risk of selection of bacterial resistance; ** Antibiotics and antibiotic classes that should be reserved for treatment of confirmed or suspected infections due to multi-drug-resistant organisms and should be treated as "last resort" options

were subjected to Gram and methylene blue staining, and 10 µl of sample was seeded on blood agar for quantitative analysis. The isolates in the number of >105 CFU/ml were further analyzed. The growth of microorganisms below the breakpoint point was considered contamination or colonization.

Bacteria were identified by conventional biochemical tests and the MALDI-TOF(matrix assisted laser desorption ionization-time of flight mass spectrometry) (Bruker, Illinois, USA) method.

The antimicrobial susceptibility to a wide range of antibiotics was determined by disk-diffusion and broth microdilution method in 96 well microtiter plates and interpreted according to CLSI breakpoints (CLSI, 2016) (Clinical Laboratory Standard institution 2016). The isolates were classified as multidrug-resistant (MDR), extensively drug resistant (XDR) or pan drug resistant (PDR) (Magiorakos et al. 2012).

Statistical Analyses

The data describing demographics, number of ventilator hours, as well as number of ICU days were summarized by the means of descriptive statistics (median, range, interquartile range due to the abnormal distribution, D'Agostino-Pearson's test).

Continuous variables were compared using Mann-Whitney's test for independent samples, while categorical variables were compared using χ-square test. The presence of Gram negative bacteria in pre-prophylactic and post-prophylactic orals swabs was compared with McNemar test for paired samples.

The sensitivity and specificity of oral swabs and tracheal aspirate in predicting the colonization of lower respiratory tract and the development of ventilator associated pneumonia were determined by receiver operating characteristic (ROC) curve analysis. The association between variables was assessed by rank correlation.

All statistical analyses were performed using MedCalc version 9.5.1.0 statistical software (MedCalc Software, Mariakerke, Belgium). P values <0.05 were considered statistically significant.

RESULTS

There were 225 patients enrolled in the study (156 males and 69 females), of median age of 65 years (range 15-93, IQR 22). Eighty-four (37.33%) patients originated from AIK, 72 (32%) from AIN, and 69 (31%) from AKA. The median stay in ICU was 8 days (1-86 days, IQR 14), with median 44 ventilator hours (range 0-1992, IQR 241). The median total length of hospital stay was 20 days (range 2-148, IQR 25). In total, 39 patients died.

The diversity of Gram- negative species found in oral swabs and in ETAs before and after the preoperative prophylactic antibiotic dose is shown in Table 2.

The number of patients colonized with Gram - negative bacteria in post-prophylactic oral swab was significantly higher in (68 of 225, 30%) compared to oral swab before prophylactic antibiotic (19 of 225, 8.4%), P<0.001, as shown in Table 3. On the other hand, the number of patients with Gram negative bacteria in ETAs (64 of 219, 29%) remained similar as in post-prophylactic oral swab (68 of 225), P=0.220 (Table 4). Moreover, we found that presence of Gram-negative bacteria in both pre- and post- prophylactic oral swabs was in the positive correlation with the presence of Gram- negative bacteria in tracheal aspirate (P, Spearman's rho, 95% CI): pre-prophylactic oral swab: P=0.042, Spearman's rho 0.128, 95% CI 0.005-0.265; post-prophylactic oral swab P<0.001, Spearman's rho 0.432, 95% CI 0.318-0.534.

Of 68 patients with Gram- negative bacteria in post-prophylactic oral swab, 37 (54%) patients had resistant bacteria (MDR or XDR) strains.

Pre-operative antibiotic prophylaxis was adequate for 102/157 (65%) patients colonized with Gram-positive bacteria in post-prophylactic oral swab and there was no need for antibiotic switch, but it was less effective in the patients colonized with Gram negative bacteria in post-prophylactic oral swab (23/68, 34%), P<0.001. Furthermore, there was positive correlation between the presence of Gram negative bacteria in post-prophylactic oral swab and the need to switch or escalate antibiotic therapy (P<0.001, Spearman's rho 0.288, 95% CI 0.163-0.403). Also, the presence of Gram- negative bacteria in post-prophylactic oral swab was associated with the need for antibiotic switch for several times: multi-switching was needed in 20/68, 29% cases with Gram- negative, compared to 16/157, 10% cases with - positive bacteria (P<0.001).

If resistant Gram- negative bacteria were present in post-prophylactic oral swab, pre-operative antibiotic prophylaxis was inadequate in 31/37 (84%) cases, compared to when sensitive strains were identified (14/31 cases), P=0.001. Also, the presence of resistant Gram- negative bacterial isolates was positively correlated with the need for antibiotic escalation (P=0.001, Spearman's rho 0.407, 95% CI 0.186-0.588). On the other hand, there was no difference in the need for multiple antibiotic switching between resistant (switching needed in 14/37 cases) and sensitive (switching needed in 7/31 cases) Gram- negative bacteria (P=0.261)

The occurrence of Gram- negative bacteria in post-prophylactic oral swab was not associated with the number of antibiotic agents given in the prophylaxis (P=0.190), nor with the WHO group of antibiotics (P=0.086).

Table 2. The diversity and the frequency of Gram- negative bacteria in oral swabs before and after single preoperative prophylactic antibiotic dose and in ETAs in 225 patients in three surgical intensive care units (AIK, AIN and AKA)

Gram- negative bacteria	Colonisation (No. of patients)		
	1st oral swab – before ATB prophylaxis*	2nd oral swab – after ATB prophylaxis*	Tracheal aspirate*,#
E. coli	4	3	4
E. coli ESBL	0	1	0
K. pneumoniae	2	3	9
K. pneumoniae ESBL	1	2	0
K. pneumoniae OXA-48	0	1	0
K. oxytoca	3	1	0
P. aeruginosa	5	33	43
C. freundii	0	2	0
C. koseri	1	0	1
A. baumani	2	14	11
A. pittii	0	0	1
E. cloacae	0	4	2
K. aerogenes	0	1	0
S. maltophilia	0	2	6
P. mirabilis	2	2	1
B. gladioli	0	1	1
S. marcescens	0	1	1
N, saprophytica	1	0	1
A. xylosoxidans	0	0	1
H. nfluenzae	0	0	5
M. catarrhalis	0	0	1
Total	*22*	*72*	*89*

ATB – antibiotic; * Several patients had multiple colonisation; # In six patients tracheal aspirate was not taken

Table 3. Gram negative microorganisms in oral swabs before and after single preoperative prophylactic antibiotic dose and in tracheal aspirates in 225 patients in three surgical intensive care units (general surgical AIK, neurosurgical-AIN, cardiosurgical-AKA)

ICU	Gram negative bacteria					
	1st oral swab – before ATB prophylaxis		2nd oral swab – after ATB prophylaxis		Tracheal aspirate*	
	Yes	No	Yes	No	Yes	No
AIK	12	72	36	48	28	51
AIN	6	66	17	55	34	37
AKA	1	68	15	54	12	69
Total	19	206	68	157	64	155

Abbreviation: ICU – intensive care unit; ATB – antibiotic; * In six patients tracheal aspirate was not taken

VAP was diagnosed in 61 patients. Out of 61 patients, 29 (47%) harboured Gram- negative bacteria in post-prophylactic oral swab, whereas resistant isolates were detected in 20 (33%) patients. Although the development of VAP was not associated with the presence of Gram- negative bacteria in pre-prophylactic oral swab (P=0.320), but there was a positive correlation with the presence of Gram- negative bacteria in post-prophylactic oral swab (P<0.001): VAP developed in only 31 of 156 patients (20%) who did not have Gram-negative bacteria in post-prophylactic oral swab, but it developed in 29 of 68 patients (43%) with Gram-negative bacteria in post-prophylactic oral swab. Also, there was positive correlation between the presence of Gram-negative bacteria in post-prophylactic oral swab and the development of VAP (P<0.001, Spearman's Rho 0.230, 95% CI 0.120-0.350). After testing for the correlation between the presence of Gram-negative bacteria in post-prophylactic oral swab and the development of VAP, we investigated sensitivity and specificity of post-

prophylactic oral swabs in the prediction of VAP. Roc curve analysis revealed that the presence of Gram-negative bacteria in post-prophylactic oral swab have moderate positive predictive value (43%, CI 33.7-51.9) and high negative predictive value (80%, 75.4-83.6) in predicting VAP (AUC 0.619, CI 0.552-0.683, z=3.273, P=0.001, sensitivity 48%, specificity 76%) (Table 4). The presence of resistant Gram- negative bacteria was associated with the development of VAP (P=0.039).

Table 4. Post-prophylactic oral swabs in the prediction of VAP in 225 patients in three surgical intensive care units (general surgical-AIK, neurosurgical-AIN, cardiosurgical-AKA)

ROC curve analysis	Post-prophylactic swab	
	VAP	Tracheal aspirate
AUC	0.619	0.708
CI	0.552-0.683	0.634 to 0.767
z	3.273	6.374
P	0.001	<0.001
Sensitivity	48%	57%
Specificity	76%	85%
PPV	43%	66
CI	33.7-51.9	55.9-75.2
NPV	80%	79
CI	75.4-83.6	74.5-83.4

Abbreviations: ROC - Receiver operating characteristic; VAP – ventilator associated pneumonia; AUC – area under the curve; CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value

As shown in Table 4, the patients in three different surgical intensive care unit differed in regard of the presence of Gram negative bacteria in the pre-prophylactic swabs (P=0.018): the patients in AIK had Gram-negative bacteria more frequently (14%) than in AIN (8%) or AKA (1%). The similar difference was found in the post-prophylactic oral swab: again, patients in AIK were more frequently colonized with Gram- negative bacteria (43%) compared to those in AIN (24%) or AKA (22%) (P=0.006). On the other hand, the Gram- negative bacteria in tracheal aspirates were found most frequently in the patients hospitalized in AIN (48%), compared to the patients in AIK (35%) and AKA (17%), P<0.001.

There was no significant difference among three surgical ICUs in the occurrence of multidrug-resistant Gram-negative bacteria (MDR) in post-prophylactic oral swab (P=0.231): MDR bacteria had 64% (23/36) of the patients in AIK, 47% (8/17) patients in AIN and 40% (6/15) in AKA. The VAP occurred the most frequently in the patients hospitalized in AIK 39% (33/84), compared to the patients in AIN 22% (16/72) and AKA 17% (12/69), P=0.005. Moreover, the frequency of VAP was correlated with the number of antibiotics given in prophylaxis. The highest rate of 42% (5/12) was observed in patients receiving three antibiotics, followed by the rate of 33% (35/105) in the patients given two prophylactic antibiotics, and only 19% (21/108) receiving only one antibiotic, P=0.038. Furthermore, VAP developed more frequently in the patients receiving two or more antibiotics from WHO Watch list (51/152), in comparison with the patients who received antibiotics form Access list (9/63), or Reserve list (1/10), P=0.007.

It was observed that prophylactic antibiotic use differed among different ICUs. Most of the patients in AIK received at least two different prophylactic antibiotics (52/84, 62%), while in AIN and AKA the majority of the patients received only one prophylactic antibiotic (47/72, 65% and 40/69, 58% respectively), P<0.001 (Table 6). This can be explained by the difference in the types of surgical procedures in different ICUs. Furthermore, while in AIN only half of the patients (33/69, 48%) received antibiotic from WHO watch group, in AIK and in AKA 64% and 86% of the patients, respectively, received antibiotic from this group, P<0.001 (Table 5).

Table 5. The use of preoperative antibiotics in 225 patients in three surgical intensive care units (general surgical, neurosurgical, cardiosurgical)

ICU	No. of patients					
	No. of ATB received			Maximal level of antibiotic received (WHO group)		
	1	2	3	Access*	Watch**	Reserve***
AIK	21	52	11	25	54	5
AIN	47	24	1	33	39	0
AKA	40	29	0	5	59	5

Abbreviation: ICU – intensive care unit; ATB – antibiotic; WHO – World Health Organization;
* Antibiotics that have activity against a wide range of commonly encountered susceptible pathogens, also showing lower resistance potential than antibiotics in the other groups and are recommended as essential first or second choice empiric treatment options;
** Antibiotic classes that have higher resistance potential and/or antibiotics that are at relatively high risk of selection of bacterial resistance; *** Antibiotics and antibiotic classes that should be reserved for treatment of confirmed or suspected infections due to multi-drug-resistant organisms and should be treated as "last resort" options

Table 6. Duration of mechanical ventilation, ICU and hospitla stay of the patients with Gram engative bacteria in per- and postprophylactic oral swab, and tracheal aspirate

Gram negative bacteria	Median, range (IQR)		
	Hours on ventilator	Days in ICU	Days in hospital
Pre-prophylactic swab			
No	39, 1-1992 (225)	8, 1-86 (13)	20, 2-148 (24)
Yes	250, 0-1236 (607)	13, 2-68 (27)	35, 2-73 (33)
P*	0.050	0.059	0.253
Post-prophylactic swab			
No	24, 1-1572 (137)	6, 1-66 (11)	18, 2-148 (18)
Yes	187, 0-1992 (611)	13, 2-86 (26)	33, 2-108 (33)
P*	<0.001	<0.001	<0.001
Tracheal aspirate			
No	17, 0-1368 (91)	6, 1-59 (7)	16, 2-148 (13)
Yes	312, 0-1992 (651)	23, 2-86 (29)	37, 4-108 (31)
P*	<0.001	<0.001	<0.001
Resistant bacteria			
No	118, 0-1440 (606)	11, 5-86 (37)	25, 2-64 (30)
Yes	288, 12.1992 (706)	15, 2-60 (23)	36, 4-108 (44)
P*	0.047	0.032	0.094

Abbreviations: IQR – interquartile range; ICU – intensive care unit; * Mann-Whitney test for independent samples

Furthermore, the ICUs differed in the need for the switch of antibiotic after obtaining the results of the susceptibility testing: the switch was needed in 59% (50/84) patients in AIK, in contrast to 42% (30/72) in AIN and 29% (20/69) in AKA (P<0.001).

The association of Gram-negative bacteria in oral swabs and ETAs with the duration of treatment and the outcome

The presence of Gram- negative bacteria in post-prophylactic oral swab and ETA significantly prolonged the duration of treatment (Table 6).

The patients with Gram-negative bacteria in post-prophylactic oral swab were 8 times longer mechanically ventilated than patients with Gram-positive bacteria (P<0.001), and had twice longer duration of ICU (P<0.001) and hospital stay (P<0.001) as shown in Table 6.

Similarly, the patients with Gram-negative bacteria in tracheal aspirate were 18 times longer mechanically ventilated than patients with Gram-positive bacteria (P<0.001), 17 days longer stay in the ICU (P<0.001), and had three weeks longer hospital stay (P<0.001).

Also, the patients with Gram-negative bacteria in ETA were 170 hours (median) longer mechanically ventilated (P=0.047), and stayed for 4 days longer in the ICU (P=0.032) compared to those without Gram-negative organism as shown in Table 6.

The presence of Gram-negative bacteria in either oral swabs or ETA was positively correlated with higher mortality. For example, 30% (7/19) of patients with Gram-negative bacteria in pre-prophylactic oral swab died compared to 16% (32/206) of patients without Gram negative bacteria in pre-prophylactic oral swab (P=0.019, Spearman's rho 0.157, 95% CI 0.0.26-0.282). The similar correlation was found for the occurrence of Gram-negative bacteria in post-prophylactic oral swab; 29% (20/68) of the patients with Gram- negative bacteria died compared to 12% (19/157) without Gram negative bacteria; P=0.002, Spearman's rho 0.210, 95% CI 0.081-0.332). The concordance was also noticed with ETAs with 31% (23/74) of the patients colonized with Gram- negative bacteria having letal exitus compared to 10% (15/145) without Gram-negative bacteria; P<0.001, Spearman's rho 0.245, 95% CI 0.116-0.365).

However, the presence of MDR Gram-negative bacteria was not correlated with the outcome: 41% (13/32) patients with MDR strain died, compared to 17% (6/36) infected with susceptible isolates (P=0.114).

Gram-negative bacteria in post-prophylactic oral swab were more frequent in the patients suffering from diabetes mellitus 48% (21/48), than in the patients without diabetes mellitus 26% (47/177), P=0.022). MDR Gram-negative bacteria in post-prophylactic oral swab were more frequent in diabetic patients compared to non-diabetic patients 81% (17/21,) vs 42% (20/47), P=0.004).

There was no correlation of diabetes mellitus and the presence of Gram-negative bacteria in ETAs (P=0.610).

DISCUSSION

The main finding of the study is that the prophylactic application of antibiotics in surgical ICUs is associated with increased colonization of oral cavity and lower respiratory tract with Gram-negative bacteria. Although the perioperative prophylaxis is based on

broad spectrum antibiotics covering both Gram-positive and Gram negative organisms, increased colonization of Gram- negative bacteria in post-prophylactic oral swab was observed. Neither the number of antibiotics in the prophylaxis, nor their level (WHO group) affected the occurrence of Gram-negative bacteria in post-prophylactic oral swab. On the contrary, the development of VAP was associated with both the number and the level of antibiotics in preoperative prophylaxis. This could be seen as a paradox at the first sight, but could be easily explained as a consequence of the irrational use of prophylactic antibiotics. Namely, since 52% of the patients received multiple antibiotics of similar coverage, and that 72% of the patients received antibiotics from Watch and Reserved group. Thus, excessive use of antibiotics form Watch and Reserved group could select resistant Gram negative-bacteria, found to be associated with the development of VAP in this and previous studies.

Moreover, the presence of the diabetes mellitus and the type of ICU affected the rate of colonization with Gram-negative bacteria. The patients in AIK had higher rates of Gram-negative bacteria in the post-prophylactic swab compared to the patients in AIN and AKA. This could be explained based on the types of surgical procedures in certain ICUs. The patients in AIK with a lot of abdominal surgical procedures had higher frequency of Gram-negative bacteria, even in pre-prophylactic swabs than the patients in AIN and AKA, which could be the consequence of more frequent vomiting (i.e. miserere) or impaired integrity of intestinal wall due to the underlying condition leading to contamination with intestinal flora. For the same reasons, this explanation could apply to post-prophylactic swabs, as well. Another explanation for this distribution could be in more contaminated surgical procedure carried out in AIK requiring more antibiotics to cover both aerobic Gram-negative enteric bacteria and anaerobes, compared to AIN and AKA which usually have “clean procedures”.

The causative agents of VAP reflected the same pattern of of Gram-negative bacteria found in oral cavity. Moreover, not only the development of VAP correlated with the presence of Gram-negative bacteria in oral cavity, but it seems that there is a high probability that VAP will develop if Gram-negative bacteria are found in post-prophylactic oral swab. In other words, post-prophylactic oral swab negative for Gram-negative bacteria could be used to predict probability of VAP development.

The limitation of the study is the small number of and the involvement of only one hospital center. However, this is the first study on the colonization of oral cavity with MDR Gram-negative bacteria and its impact on development of hospital pneumonia. The risk factors for development of VAP need to be identified and clarified in order to prevent and control this important complication of mechanical ventilation.

CONCLUSIONS

This study showed that Gram- negative bacteria in oral swabs occured more frequently after preoperative prophylactic antibiotics. Furthermore, receiving two prophylactic antibiotics was associated with the higher incidence of Gram negative bacteria in oral swabs and ETAs, and that VAP itsels developed more frequently in the patients receiving two or more antibiotics from WHO Watch list. Therefore, we pledge here for the rational use of antibiotics in preoperative prophylaxis.

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Contribution of individual authors:

Vesna Bratić: design of the study, literature searches and analyses, interpretation of data, manuscript writing.

Anita Lukić: data collection, statistical analysis.

Branka Bedenić: design of the study, manuscript writing.

Slobodan Mihaljević: design of the study, literature searches and analyses.

Željko Verzak: design of the study, manuscript writing.

All authors have read and agreed to the published version of the manuscript.

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