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Clindamycin-resistant *Clostridioides difficile*: a challenge in dentistry

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ABSTRACT

Introduction: Clindamycin is frequently prescribed in dentistry. The spread of clindamycin resistance among *Clostridioides difficile* strains and associated severe side effects have triggered patients' concern. The extent of this concern and how it is addressed were investigated in this study. Pro- or synbiotics have been suggested to prevent antibiotic-induced *C. difficile* infection (CDI). *In vitro* experiments were performed to evaluate what role these products can play in managing clindamycin-resistant CDI and which product features might be beneficial.

Material and methods: A survey among German dentists evaluated the importance of side effects of antibiotics during discussions with patients, clindamycin usage and whether co-administration of pro- or synbiotics is recommended. Three different *C. difficile* strains were characterized by antibiotic susceptibility testing (AST) and the possibility to inhibit their in vitro growth by products containing probiotic microorganisms.

Results: All respondents claimed that side effects of antibiotics are a topic of discussion with patients, 92% reported using clindamycin. 6% of respondents stated that they did not recommend pro- or synbiotics, while 67% claimed to make this recommendation with each antibiotic prescription. AST of the three investigated *C. difficile* strains revealed resistance against clindamycin (and other antibiotics) of the *C. difficile* ribotype 001 strain No. 977 and the ribotype 027 strain No. 644. In vitro inhibition experiments showed that all three strains could be best inhibited by multi-strain synbiotic preparations.

Conclusions: The recommendation to co-administer pro- or synbiotics together with clindamycin can be used to address concerns of patients. The results of this study support this approach and provide some guidance for product selection.

KEY WORDS: clindamycin, *Clostridioides difficile*, gut-microbiome, multi-drug resistance, PCR ribotype 027.

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INTRODUCTION

Clindamycin is a broad-spectrum antibiotic commonly used in dentistry for the treatment of odontogenic infections (e.g., periapical abscess, pericoronitis, and periodontal abscess) as well as the prophylaxis of infective endocarditis. Odontogenic infections are mostly polymicrobial, comprising facultative anaerobes (e.g., *Streptococci* spp.) and strict anaerobes (e.g., *Prevotella* spp., *Fusobacterium* spp.) [1]. Clindamycin's antibacterial profile covers this spectrum of bacterial pathogens excellently [2]. In addition to its anti-infective properties, clindamycin has high oral absorption, significant tissue penetration and good penetration into bone [3, 4]. Common side effects of systemic clindamycin therapy (affecting > 1% of patients) are diarrhea, nausea, vomiting, abdominal pain, cramps and/or a rash [5]. In some

patients clindamycin therapy can result in the development of potentially lethal pseudomembranous colitis (PMC), which is caused by overgrowth of the gut by the bacterial pathogen *Clostridioides difficile* [6]. Most *C. difficile* strains, among them the hypervirulent epidemic PCR-ribotype 027 (also known as North American Pulsotype 1 [NAP1] or restriction endonuclease analysis [REA] BI type [BI]) strains, have become clindamycin-resistant [7]. As the majority of the beneficial bacteria in the gut are still susceptible to clindamycin, the therapy with this antibiotic provides clindamycin-resistant *C. difficile* strains with a strong growth advantage in the gut. By using this growth advantage, the clindamycin-resistant *C. difficile* strains can overcome the colonization resistance normally provided by a diverse and balanced bacterial gut microbiota [8-10]. Because of the associated risk of PMC and the spread of resistance among *C. difficile* strains, prescription of clindamycin should follow a good antibiotic stewardship. At the same time, usage of preparations containing probiotic microorganisms (probiotics or synbiotics) as prophylactic CDI measures, at least for patients at risk (e.g., the elderly or those with previous CDI), and as complementary therapy during and after clindamycin therapy, is receiving growing interest. Results from a number of studies found evidence that probiotics or synbiotics could play a role in the management of CDI. *In vitro* growth inhibition of *C. difficile* has been demonstrated for mono-strain probiotics [11, 12] and multi-strain pro- or synbiotics [13]. Administration of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* seemed to have a neutralizing effect on the toxins of *Clostridioides difficile*, as it was found that only 46% of patients who received the probiotic were toxin-positive, compared to 78% of patients in the placebo group [14]. Colonization of epithelial cells by *C. difficile* could be prevented by administering a mixture of *Staphylococcus*, *Enterococcus*, *Lactobacillus*, *Anaerostipes*, *Bacteroidetes*, and *Enterorhabdus* [15]. The probiotic yeast *Saccharomyces boulardii* upregulated the expression of anti-TcdA secretory immunoglobulin A in animal models of CDI and inhibited the binding of TcdA to epithelial cells [16, 17]. A mixed culture of non-toxigenic *Clostridioides difficile*, *Escherichia coli*, *Bifidobacterium bifidum*, and members of *Lachnospiraceae* was found to prevent the colonization of *Clostridioides difficile* in germ-free mice [18, 19]. A meta-analysis [20] showed that probiotics are associated with a reduction in the incidence of CDI-associated diarrhea. A review published in 2008 by the Cochrane Group [21] stated that there were still not enough data to establish the role of probiotics for the treatment of CDI. However, in a more recent systematic review and meta-analysis by the Cochrane Collaboration, published in 2017, the authors concluded that probiotics have general positive effects in CDI patients [22]. While there are currently not sufficient data to support a positive recommendation to use products containing probiotic microorganisms for the management of CDI, these products are known to be used by dentists, although to what extent is not well characterized.

Anecdotal evidence collected by the authors over recent years indicates that patients of dentists increasingly demand to discuss side effects of antibiotic therapy, especially the potential problems related to clindamycin therapy. Some dentists have even reported that compliance of patients has been negatively affected by this concern. The present study had two objectives: Firstly, a survey was carried out among German dentists to investigate the extent to which antibiotic side effects are a topic during routine patient/dentist discussions, assess the utilization rate of clindamycin in dentists' daily routine and determine to what extent and for what purpose dentists recommend probiotics or synbiotics when prescribing clindamycin or other antibiotics. In the second, experimental part of the study, the antibiotic susceptibilities of three different *Clostridioides difficile* strains (*C. difficile* [ATCC 9689], *C. difficile* No. 977 ribotype 001 and *C. difficile* No. 644 PCR ribotype 027) were characterized. *In vitro* pathogen inhibition experiments were employed to determine whether the growth of these different *C. difficile* strains could be inhibited by products containing probiotic microorganisms. Despite the obvious limitations of results from *in vitro* experiments, the present results can provide some guidance to dentists when making recommendations to patients to co-administer probiotics and synbiotics while taking clindamycin or other antibiotics.

MATERIAL AND METHODS

A cross-sectional study employing a survey among German dentists was performed by sending a short cover letter outlining the objectives of the research project and a one-page questionnaire comprising four questions (Table 1) by regular post.

Postal addresses for German dentists were taken from a commercially available database. Dentists were provided with a national fax number to which they were invited to send the completed questionnaire. One reminder was sent to dentists who had not responded within two weeks after the first contact. Data processing was approved by respondents by stamp, date and their signature.

Only questionnaires with answers to all questions were analyzed. Survey answers were collected until the data from the last ten newly collected questionnaires did not change the percent values to the predefined answers of question three by more than 3%. The maximum percentage change caused by the last ten collected questionnaires was actually 2.4%.

The yeast probiotic Enterol (Biocodex, Gentilly, France) contains in each capsule 4.5×10^9 colony forming units (CFU) of the *Saccharomyces boulardii* strain CNCM I-745. Dicoflor (Bayer Sp. z o.o., Warszawa, Poland) contains 6×10^9 CFU of *Lacticaseibacillus rhamnosus* GG

TABLE 1. Questions of the survey

No	Question	Type of answer
1	Does the topic of side-effects of antibiotics come up in discussions with your patients?	Selection of one of the pre-defined answers
2	Which antibiotic are you using in your daily routine?	Multiple selection of predefined answers and field for free-text answer
3	Do you recommend the co-administration of probiotics or synbiotics when you prescribe antibiotics?	Selection of one of the pre-defined answers
4	Are you interested in information about *Clostridioides difficile*?	Selection of one of the pre-defined answers

ATCC 53103 per capsule. The multi-strain synbiotic A (Vivatlac, Vivatrex GmbH, Rees, Germany) contains in each capsule 9.0×10^8 *Lactococcus lactis Ll-23*, 9.0×10^8 CFUs *Lactobacillus helveticus SP 27*, 6.75×10^8 CFUs *Bifidobacterium longum Bl-05*, 4.5×10^8 CFUs *Bifidobacterium breve Bb-03*, 4.5×10^8 CFUs *Lacticaseibacillus rhamnosus Lr-32*, 4.5×10^8 CFUs *Streptococcus thermophilus St-21*, 2.25×10^8 CFUs *Lacticaseibacillus casei Lc-11*, 2.25×10^8 CFUs *Lactiplantibacillus plantarum Lp-115*, 2.25×10^8 CFUs *Bifidobacterium bifidum Bb-02*, and 68 mg of the prebiotic FOS. The multi-strain synbiotic B (Vivatlac Baby, Vivatrex GmbH, Rees, Germany) is a freeze-dried powder. Each sachet contains a total of 10^9 CFUs as a mixture of equal CFU amounts of *Lactobacillus acidophilus LA-14*, *Lacticaseibacillus casei R0215*, *Lacticaseibacillus paracasei Lpc-3*, *Lactiplantibacillus plantarum Lp-115*, *Lacticaseibacillus rhamnosus GG*, *Ligilactobacillus salivarius Ls-33*, *Bifidobacterium lactis Bl-04*, *Bifidobacterium bifidum R0071*, *Bifidobacterium longum R0175* and 1.43 g of the prebiotic FOS.

The ribotype 001 strain *C. difficile* (ATCC 9689) was purchased from ATCC, Manassas, Virginia, USA [23]. The bacterial strains *C. difficile* No. 644 and *C. difficile* No. 977 are members of a collection of *C. difficile* strains that has been established in the course of a surveillance study conducted in 2012 to obtain an overview of CDI in Polish hospitals [24]. Ethical approval and informed consent were not required. The strains were isolated from CDI patients, diagnosed on the basis of the CDI definitions of 2012 proposed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) [25]. For isolation of the strains, the fecal sample was inoculated anaerobically on selective media for 48 h, and *C. difficile* colonies were sub-cultured on blood-agar and identified using standard methods, as described previously [26]. PCR ribotyping of the isolates was performed by the Anaerobe Laboratory, Medical University of Warsaw according to the method described by Stubbs *et al.* [27]. The Cardiff-ECDC collection of reference isolates ($n = 23$) of *C. difficile* was used as a reference set.

AST (antimicrobial susceptibility testing) of the *C. difficile* strains was performed using the gradient diffusion method ETEST for epidemiological research (bioMérieux SA, Marcy l'Etoile, France). Tests with ETEST strips that contained gradients of each agent tested were performed as specified by the producer [28]. The following antimicrobials were tested: metronidazole (MZ) and vancomycin (VA), clindamycin (CLI), erythromycin (ERY), which had ETEST strips ranging from 0.016 to 256 mg/l; and ciprofloxacin (CIP), moxifloxacin (MXF), imipenem (IP), which had ETEST strips ranging from 0.002 to 32 mg/l. Minimum inhibitory concentration (MIC) values were read from the scales in terms of mg/l at complete inhibition of growth of the respective *C. difficile* strain. European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints for *C. difficile* were applied to the antimicrobial drugs MXF, MZ and VA (http://www.eucast.org) [29]. For CIP, CLI, ERY and IP, Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints were assessed [30].

For the *in vitro* pathogen inhibition studies with the different *C. difficile* strains, the pathogens were cultivated under anaerobic conditions at 35-37°C for 24-48 h on Schaedler agar (CM0437, Fisher Scientific GmbH, Schwerte, Germany) [31]. Suspensions of the evaluated products each containing 10^6 CFU were inoculated on MRS agar and incubated for 48 h in the presence of 5% CO_2. 10 mm diameter bars were transferred to a Mueller-Hinton agar with 5% horse blood and 20 mg/l NAD (PP0972, E&O Laboratories Ltd, Bonnybridge, UK) and incubated under anaerobic conditions for 24 h.

For testing a potential pathogen growth inhibitory effect of FOS, 100 µl of a solution containing 14.3 mg/ml FOS (F8052, Sigma Aldrich, St. Louis, Missouri, USA) was applied to a 10 mm filter disk that was then administered to respective pathogen testing plates. The multi-strain synbiotics A and B containing nine different probiotic strains were tested on the same plates as positive controls.

At the end of the incubation, measurements of inhibition zones around the tested colonies were taken from the outer edge of the colonies to the outer edge of the clear zones. Each test was performed in triplicate and the arithmetic means of the radii measuring from the edges of the colonies to the edges of the clear zones were calculated, as well as the standard deviations SD (Excel, Microsoft, Redmond, Washington, USA). Independent *t*-test statistical analyses of datasets were conducted

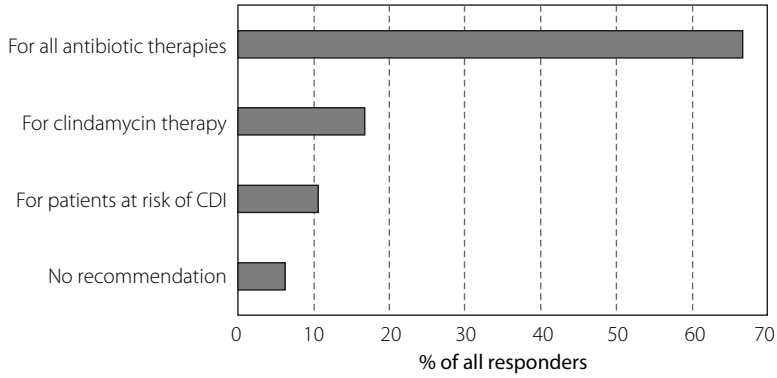

FIGURE 1. Recommendation of co-administration of probiotics and synbiotics by German dentists ($n = 66$)

with GraphPad Prism software version 8.2 (GraphPad Software, San Diego, California, USA). Datasets were considered as significantly different when a p-value < 0.01 was achieved.

RESULTS

From September 2020 to January 2021, 633 German dentists were contacted. Responses from a total of 68 (response rate 10.7%) were collected, of which two (2.9% of all respondents) were incomplete and therefore were excluded from further analysis. All responding dentists (100%) stated that the topic "side effects of antibiotics" is part of their communication with patients and nearly all dentists (92%) reported using clindamycin as part of their daily antibiotic routine. Only 6% of the responding dentists claimed not to recommend probiotics or synbiotics to their patients when prescribing antibiotics (Figure 1). Nearly two thirds of dentists stated that they recommend the co-administration of pro- or synbiotics for all antibiotic therapies they prescribe. The remaining 27% of respondents reported a more selective recommendation behavior: 16.7% claimed to make such a recommendation only when prescribing clindamycin and 10.6% stated that they based their recommendation on a CDI risk assessment of their patients.

Interest to receive more information about *C. difficile* was expressed by 79% of all respondents.

AST was used to investigate the resistance profile of the three investigated *C. difficile* strains (Table 2). Minimum inhibition concentrations (MICs) above the respective resistant breakpoint concentration were rated as resistant (R), those below as susceptible (S), and those equal to the respective resistant breakpoint concentration as medium susceptible (MS). Resistance breakpoints for metronidazole, vancomycin and moxifloxacin were taken from the most recent publication of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [29], those for clindamycin, erythromycin, ciprofloxacin and imipenem from the actual publication of the Clinical and Laboratory Standards Institute (CLSI) [30].

The reference strain *C. difficile* (ATCC 9689) exhibited resistance against fluoroquinolones and a somewhat reduced susceptibility against imipenem. In contrast, *C. difficile* No. 977 and *C. difficile* No. 644 were multidrug resistant, defined as being resistant against antibiotics from at least three different antibiotic classes. Both strains demonstrated resistance to clindamycin, erythromycin, the fluoroquinolones ciprofloxacin and moxifloxacin as well as the carbapenem antibiotic imipenem.

TABLE 2. Antimicrobial susceptibility of *Clostridioides difficile* strains

Antibiotic	*C. difficile* (ATCC 9689)	*C. difficile* No. 977	*C. difficile* No. 644
PCR-Ribotype	001	001	027
Clindamycin[1]	S	R	R
Erythromycin[2]	S	R	R
Ciprofloxacin[2]	R	R	R
Moxifloxacin[3]	R	R	R
Imipenem[2]	MS	R	R
Metronidazole[1]	S	S	S
Vancomycin[1]	S	S	S

R: resistant (MIC values ≥ resistance breakpoint concentration), S: susceptible (MIC values ≤ resistance breakpoint concentration), MS: medium susceptible (MIC values equal to resistance breakpoint concentrations).
[1]Resistance breakpoint concentration taken from EUCAST.
[2]Resistance breakpoint concentration taken from CLSI.
[3]Epidemiological cut-off concentration taken from EUCAST.

Results of the *in vitro* growth inhibition experiments are shown in Figure 2. The inhibitory effects of each tested product on the three different *C. difficile* strains were similar; in none of the cases was a significant difference (p-value > 0.05) found. In contrast, there were significant differences when the inhibitory effects of the different products containing probiotic microorganisms were compared. The weakest inhibitory effects were determined for the product containing the yeast probiotic *Saccharomyces boulardii*. The mono-strain bacterial probiotic containing *Lacticaseibacillus* (L.) *rhamnosus GG* caused medium size inhibition. The multi-strain bacterial synbiotics caused the strongest inhibition. There were no significant differences (p-value > 0.05) between the

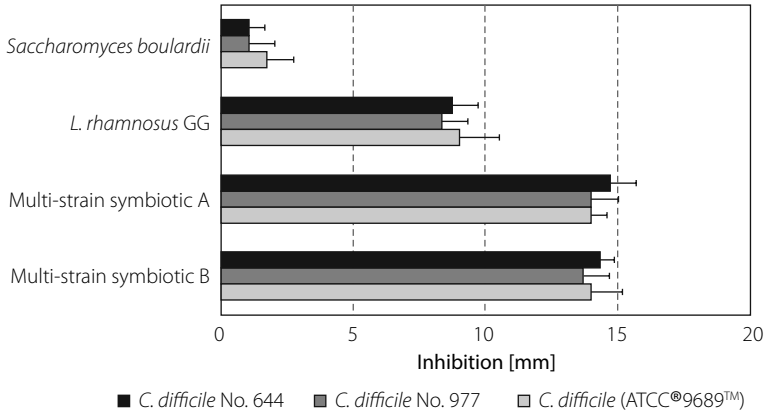

FIGURE 2. *In vitro* growth inhibition of *C. difficile* strains by the yeast probiotic *Saccharomyces boulardii*, the bacterial probiotic *L. rhamnosus* GG and two bacterial multi-strain synbiotics. Detailed information about the composition of the multi-strain synbiotics is provided under Materials and methods

inhibitory effects of the two multi-strain synbiotics. FOS alone caused no inhibition of the growth of the *C. difficile* strains (data not shown).

DISCUSSION

Results of the survey revealed that discussion about the adverse events of antibiotic therapy is a common topic encountered by the responding dentists when they speak with their patients. High percentages of the respondents claimed to use clindamycin and to recommend the co-administration of probiotics or synbiotics when they prescribe antibiotics. The results from the survey have to be interpreted with caution. Firstly, the survey response rate was only slightly above 10%. This relatively low response rate limits the possibility to generalize the findings of the survey. Secondly, the respondents might be especially interested in the topic, while dentists who are not interested in the subject may have tended not to participate. In that case, it is possible that the topic addressed in the questionnaire has activated only a specific subset of dentists. Consequently, the survey results might not be representative of the attitude of all dentists. Therefore, the actual usage rate of clindamycin by all German dentists, as well as the rate of recommendation to co-administer probiotics or synbiotics, might be significantly lower than the rates found in this study. In the worst case, only users of clindamycin and dentists recommending the co-administration of probiotics or synbiotics might have responded to the survey. This would mean that only about 10% of dentists are using clindamycin and are performing pro- or synbiotic co-administration recommendations. Even then, the topic of clindamycin side effects and the possibility to alleviate them by the administration of probiotic or synbiotics remains an important issue for a large number of dentists. Despite the inherent limitations of the survey, it is an interesting finding that two thirds of respondents stated that they recommend the co-administration of probiotics or synbiotics to all patients independent of the type of antibiotic prescribed. 16.7% of respondents claimed to make such a recommendation only when they prescribe clindamycin and another 10.6% based this recommendation on a CDI risk assessment of the patient to whom they prescribe an antibiotic.

The results from antibiotic susceptibility testing performed for the three *C. difficile* strains are in line with findings of others, indicating that multi-drug resistance is a common feature of *C. difficile* strains [32-34]. As of today, resistance to clindamycin, erythromycin and quinolones is a common finding for *C. difficile* strains isolated from CDI patients around the world. Between 1 and 4% of the general population are assumed to be symptom-free carriers of *C. difficile* [35]. Significantly higher carrier rates have been found in patients with acute or recent exposure to healthcare and in the elderly [36, 37]. Consequently, a significant percentage of patients treated by dentists might be carriers of *C. difficile*. Dentists have to keep this in mind, as prescribing an antibiotic to a *C. difficile* carrier can unleash this pathogen, with sometimes fatal consequences. A diverse and balanced bacterial gut microbiota has been found to provide protection against overgrowth of the gut by *C. difficile*, and the administration of products containing probiotic microorganisms therefore might be a sensible approach to keep this pathogen at bay [22]. Unfortunately, data from clinical studies investigating this potential effect of pro- or synbiotics are limited, as most modern probiotics and synbiotics are food supplements which do not seem to be of too much interest to the pharmaceutical industry. Despite the lack of strong evidence-based support for the usage of probiotics or synbiotics for the management of CDI, the majority of respondents of this study's survey have already adopted this approach. Characterization of the types of products recommended by dentists and the underlying drivers for product selection has to be evaluated in a future study. There is a large number of different probiotic and synbiotic products available on the market, and product selection could be a challenge.

Products containing probiotic microorganisms can be differentiated by the type of probiotic (yeast or bacteria), the number of probiotic strains (mono-strain or multi-strain) and the presence (synbiotic) or absence (probiotic) of a prebiotic component.

The present study compared the *C. difficile* growth inhibitory effects of a mono-strain probiotic containing the yeast *Saccharomyces boulardii*, a mono-strain bacterial probiotic containing *L. rhamnosus*, and two multi-strain bacterial synbiotics which contain FOS as a prebiotic component. Inhibition rates observed were not *C. difficile* strain dependent, an effect that has also recently been demonstrated for different strains of *Klebsiella pneumoniae* [38] and for *Salmonella enterica typhimurium* [39]. The *C. difficile* growth inhibition observed for the multi-strain synbiotics was found to be significantly stronger than that observed for the mono-strain probiotics. A potential reason for the superior effects of the multi-strain synbiotics might be synergistic effects among the different probiotic bacteria, leading to a stronger overall inhibitory effect on the growth of *C. difficile* [40-42].

CONCLUSIONS

Patients of dentists are increasingly aware of side effects of antibiotics and clindamycin in particular. A solid knowledge of clindamycin's potential side effects, the role of *C. difficile* in these adverse actions, and the possibility to alleviate the effects by co-administration of products containing probiotic microorganisms will help dentists to address this concern of patients. Results from *in vitro C. difficile* growth inhibition experiments allow one to differentiate among the large number of products present on the market and can guide product selection, at least as long as data from clinical studies remain unavailable.

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DISCLOSURE

The authors report no conflict of interest.

References

1. Robertson D, Smith AJ. The microbiology of the acute dental abscess. J Med Microbiol 2009; 58(Pt 2): 155-162.
2. Brook I, Lewis MA, Sándor GK, et al. Clindamycin in dentistry: more than just effective prophylaxis for endocarditis? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 100(5): 550-558.
3. Nicholas P, Meyers BR, Levy RN, Hirschman SZ. Concentration of clindamycin in human bone. Antimicrob Agents Chemother 1975; 8(2): 220-221.
4. Thabit AK, Fatani DF, Bamakhrama MS, et al. Antibiotic penetration into bone and joints: an updated review. Int J Infect Dis 2019; 81: 128-136.
5. Clindamycin side effects. Available from: https://www.drugs.com/sfx/clindamycin-side-effects.html (accessed: 11 January 2022).
6. Farooq PD, Urrunaga NH, Tang DM, von Rosenvinge EC. Pseudomembranous colitis. Dis Mon 2015; 61(5): 181-206.
7. Banawas SS. Clostridium difficile Infections: a global overview of drug sensitivity and resistance mechanisms. Biomed Res Int 2018; 2018: 8414257.
8. Ducarmon QR, Zwittink RD, Hornung BVH, et al. Gut microbiota and colonization resistance against bacterial enteric infection. Microbiol Mol Biol Rev 2019; 83(3): e00007-19.
9. Kim S, Covington A, Pamer EG. The intestinal microbiota: antibiotics, colonization resistance, and enteric pathogens. Immunol Rev 2017; 279(1): 90-105.
10. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 2013; 13(11): 790-801.
11. Naaber P, Smidt I, Štšepetova J, et al. Inhibition of Clostridium difficile strains by intestinal Lactobacillus species. J Med Microbiol 2004; 53(Pt 6): 551-554.
12. Schoster A, Kokotovic B, Permin A, et al. In vitro inhibition of Clostridium difficile and Clostridium perfringens by commercial probiotic strains. Anaerobe 2013; 20: 36-41.
13. Piatek J, Krauss H, Ciechelska-Rybarczyk A, et al. In-vitro growth inhibition of bacterial pathogens by probiotics and a synbiotic: product pomposition patters. Int J Environ Res Public Health 2020; 17(9): 3332.
14. Plummer S, Weaver MA, Harris JC, et al. Clostridium difficile pilot study: effects of probiotic supplementation on the incidence of C. difficile diarrhoea. Int Microbiol 2004; 7(1): 59-62.
15. Lawley TD, Clare S, Walker AW, et al. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice. PLoS Pathog 2012; 8(10): e1002995.
16. Buts JP, De Keyser N, De Raedemaeker L. Saccharomyces boulardii enhances rat intestinal enzyme expression by endoluminal release of polyamines. Pediatr Res 1994; 36(4): 522-527.
17. Qamar A, Aboudola S, Warny M, et al. Saccharomyces boulardii stimulates intestinal immunoglobulin A immune response to Clostridium difficile toxin A in mice. Infect Immun 2001; 69(4): 2762-2765.
18. Corthier G, Dubos F, Raibaud P. Modulation of cytotoxin production by Clostridium difficile in the intestinal tracts of gnotobiotic mice inoculated with various human intestinal bacteria. Appl Environ Microbiol 1985; 49(1): 250-252.
19. Reeves AE, Koenigsknecht MJ, Bergin IL, Young VB. Suppression of Clostridium difficile in the gastrointestinal tracts of germfree mice inoculated with a murine isolate from the family Lachnospiraceae. Infect Immun 2012; 80(11): 3786-3794.
20. Lau CS, Chamberlain RS. Probiotics are effective at preventing Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. Int J Gen Med 2016; 9: 27-37.
21. Pillai A, Nelson R. Probiotics for treatment of Clostridium difficile-associated colitis in adults. Cochrane Database Syst Rev 2008; (1): CD004611.

22. Goldenberg JZ, Yap C, Lytvyn L, et al. Probiotics for the prevention of Clostridium difficile-associated diarrhea in adults and children. Cochrane Database Syst Rev 2017; 12(12): CD006095.
23. Clostridioides difficile (Prevot) Lawson et al. (ATCC® 9689™). Available from: https://www.atcc.org/products/all/ 9689.aspx#documentation (accessed: 11 January 2022).
24. Lachowicz D, Pituch H, Obuch-Woszczatyński P. Antimicrobial susceptibility patterns of Clostridium difficile strains belonging to different polymerase chain reaction ribotypes isolated in Poland in 2012. Anaerobe 2015; 31: 37-41.
25. Kuijper EJ, Coignard, B, Tüll P; ESCMID Study Group for Clostridium difficile; EU Member States; European Centre for Disease Prevention and Control. Emergence of Clostridium difficile-associated disease in North America and Europe. Clin Microbiol Infect 2006; 12 Suppl 6: 2-18.
26. Obuch-Woszczatyński P, Lachowicz D, Schneider A, et al. Occurrence of Clostridium difficile PCR-ribotype 027 and it's closely related PCR-ribotype 176 in hospitals in Poland in 2008-2010. Anaerobe 2014; 28: 13-17.
27. Stubbs SL, Brazier JS, O'Neill GL, Duerden BI. PCR targeted to the 16S-23S rRNA gene intergenic spacer region of Clostridium difficile and construction of a library consisting of 116 different PCR ribotypes. J Clin Microbiol 1999; 37(2): 461-463.
28. ETEST® product brochure. Available from: https://www.biomerieux-usa.com/sites/subsidiary_us/files/prn_056750_rev_01.a_cln_idast_etest_brochure_final_art_0.pdf (accessed: 11 January 2022).
29. EUCAST: European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoints and dosing of antibiotics. Available from: https://www.eucast.org/clinical_breakpoints/ (assessed: 11 January 2022).
30. Carpenter DE, Anderson K, Citron DM, et al. Methods for antimicrobial susceptibility testing of anaerobic bacteria. CLSI standard M11. 9th ed. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2018, pp. 1-47.
31. Schaedler RW, Dubos R, Costello R. The development of the bacterial flora in the gastrointestinal tract of mice. J Exp Med 1965; 122: 59-66.
32. Peng Z, Jin D, Kim HB, et al. Update on antimicrobial resistance in Clostridium difficile: resistance mechanisms and antimicrobial susceptibility testing. J Clin Microbiol 2017; 55(7): 1998-2008.
33. Tenover FC, Tickler IA, Persing DH. Antimicrobial-resistant strains of Clostridium difficile from North America. Antimicrob Agents Chemother 2012; 56(6): 2929-2932.
34. Goudarzi M, Goudarzi H, Alebouyeh M, et al. Antimicrobial susceptibility of clostridium difficile clinical isolates in iran. Iran Red Crescent Med J 2013; 15(8): 704-711.
35. Galdys AL, Curry SR, Harrison LH. Asymptomatic Clostridium difficile colonization as a reservoir for Clostridium difficile infection. Expert Rev Anti Infect Ther 2014; 12(8): 967-980.
36. Truong C, Schroeder LF, Gaur R, et al. Clostridium difficile rates in asymptomatic and symptomatic hospitalized patients using nucleic acid testing. Diagn Microbiol Infect Dis 2017; 87(4): 365-370.
37. Nissle K, Kopf D, Rösler A. Asymptomatic and yet C. difficile-toxin positive? Prevalence and risk factors of carriers of toxigenic Clostridium difficile among geriatric in-patients. BMC Geriatr 2016; 16(1): 185.
38. Piatek J, Bernatek M, Ciechelska-Rybarczyk A, et al. Inhibition of carbapenem-resistant NDM-1 Klebsiella pneumoniae isolated from a hospital outbreak patient by a synbiotic: a nonantibiotic treatment option. Int J Med Res Health Sci 2019; 8(9): 12-20.
39. Piatek J, Sommermeyer H, Bernatek M, et al. Persistent infection by Salmonella enterica servovar typhimurium: are synbiotics a therapeutic option? – a case report. Benef Microbes 2019; 10(2): 211-217.
40. El Hage R, Hernandez-Sanabria E, Van de Wiele T. Emerging trends in "smart probiotics": functional consideration for the development of novel health and industrial applications. Front Microbiol 2017; 8: 1889.
41. Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 2017; 9(9): 1021.
42. Timmerman HM, Koning CJ, Mulder L, et al. Monostrain, multistrain and multispecies probiotics – comparison of functionality and efficacy. Int J Food Microbiol 2004; 96(3): 219-233.

AUTHORS' CONTRIBUTIONS

HS, JP prepared the concept of the paper. HP collected data. DW, ZK, PWB, MB analysed data. HS, MB wrote the article. All authors have given their approval to the final version of the paper.