Clostridium difficile associated infection in Russia: epidemiology, virulence, pathophysiological aspects

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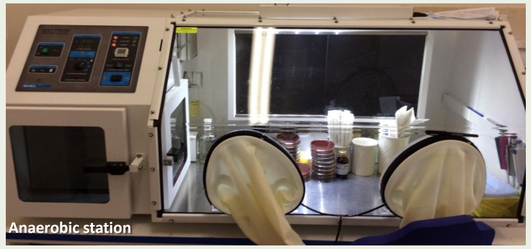
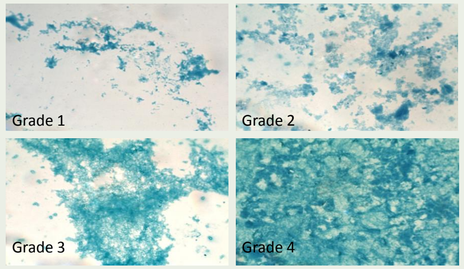
Background

Antibiotic-associated diarrhea (AAD) is the most frequent complication of antibiotic treatment. *Clostridium difficile* (CD) is a main cause of AAD due to toxins A (toxA) and B (toxB); herewith spore and biofilm formation hinders specific treatment and promotes disease recurrence. Thus, our aim was to investigate the spread and etiological structure of CD infection (CDI) as well as pathogenicity factors and some aspects of CD-driven host immune response in Russian population.

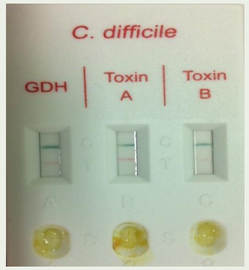


Material/methods

Biofilm formation (alcian blue , × 960)



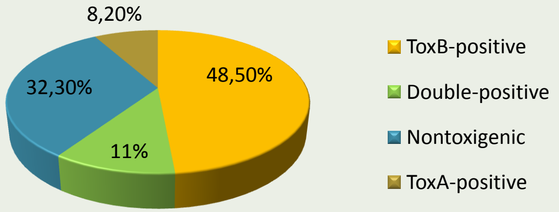
Luminal faeces screening



A total of 522 patients from coloproctological hospital participated in this research. The luminal faeces were screened for glutamatdehydrogenase (GDH), toxA and toxB. Bateriological assay was also held in all cases. Isolated strains were tested for pathogenicity factors: haemolysin, toxins, biofilms and antibiotic resistanse. 15 patients with clinical manifestation of CDI and 16 asymptomatic CD carriers participated in the assessment of biofilm formation. Biofilms were grown on glass in a 2 hour time interval with subsequent fixation in 96% ethanol and staining with alcian blue or calcofluor. Biofilm samples were analyzed by means of fluorescent and conventional microscopy. Biofilms were scored to grades 0 – 4 according to their surface. We also investigated oxidative output of neutrophils and monocytes from the whole blood of 10 healthy volunteers. Phagocytes were incubated with 4 CD strains (one clinical manifestation (508) and 3 carriers) and dihydrorhodamine (DHR) 123 for 30 minutes at 37° and then rhodamine (Rho) 123 positive cells were quantified by flow cytometry.

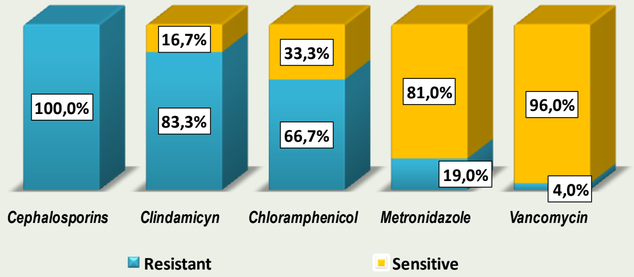
Results

Structure of C. difficile toxin production



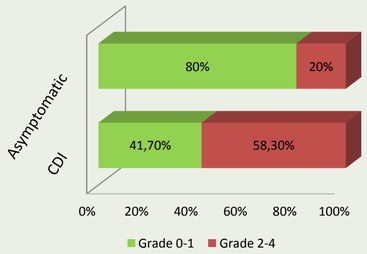
CD was isolated in 53% of all cases, 67,7% of them were toxigenic : toxB-positive in 71,7%, toxA-positive in 12,1% and double-positive in 16,2% of cases. All isolated cultures produced haemolysins.

Clostridium difficile antibiotic resistance



Toxigenic CD strains were resistant to cephalosporins in 100%, clindamicyn in 83,3%, chloramphenicol in 66,7%, metronidazole in 19,7%, vancomycin in 4% of cases.

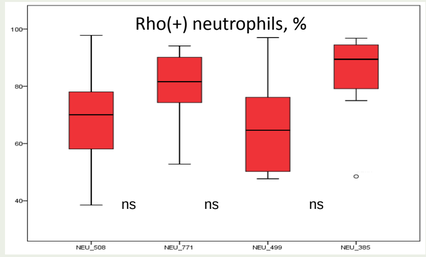
Biofilm formation

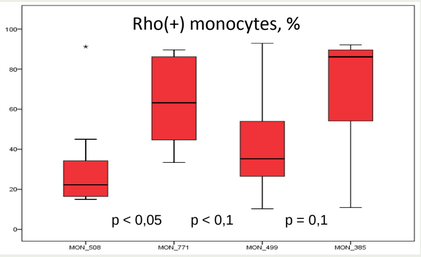


56 CD strains were tested for biofilm formation. 69,2% of strains showed high intensity (grade 3-4) of biofilm formation. Moreover, isolates from patients with clinical manifestation CDI showed significantly ($p<0,05$) higher intensity of biofilm production compared to asymptomatic CD carriers.

Phagocyte oxidative output

Our analysis showed no differences in neutrophil reactive oxygen species production under stimulation by different CD strains, however oxydative metabolism of monocytes was significantly ($p < 0,05$) lower in system stimulated by CD strain from patient with CDI (22,3% of positive cells versus 63,2%, 32,3% and 86,1% in asymptomatic carriers). All strains showed strong significant correlation between neutrophil and monocyte oxidative output.





Strain	Pearson's η (Neu / Mon)	p value
508	0,736	0,024
771	0,804	0,005
499	0,828	0,003
385	0,767	0,016

Conclusions

1. Spread of CD is an emerging clinical challenge in Russian population
2. Toxin B is the main virulence factor of CD
3. High level of antibiotic resistance determines the importance of adequate antibiotic therapy for CDI
4. Biofilm formation is an important pathogenicity factor of CD. Clinically significant CDI is associated with higher rate of biofilm production
5. Lower intensity of phagocytes' oxidative metabolism induced by CD strain from a patient with clinically active CDI provides an evidence of an impaired phagocytes' function that leads to infection progression