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Diarrheal diseases and molecular detection of bacterial enteropathogens from hospitalized cases of diarrhea

Diarrheal diseases

Morbidity from diarrheal diseases are common among people residing in developing and underdeveloped countries. The disease is the 2nd leading cause of childhood mortality and is globally responsible for the cause of 760,000 deaths of children under five years of age. India contributes to about 77% of the child deaths in South-east Asia and 18% of the global child deaths due to diarrhea. The irony lies in the fact that most diarrheas are treatable and most of the diarrheal deaths are preventable. Diarrhea should thus be attended rapidly and effectively to detect the causal etiology and to avoid significant morbidity and mortality and also to prevent secondary transmission.

The prime cause and spread of the disease is due to compromised hygiene and lack of access to safe drinking water. Intervention measures primarily included access to have improved hygiene, safe drinking water and to practice handwashing. Such measures will definitely cut down the occurrence of diarrhea as the disease spread through ingestion of contaminated food or water, or from person to person contact aided by lack of proper hygiene. Under recently initiated 'Swachh Bharat' programme, India is looking forward to have 'total sanitation' by 2019 which is promise for having a toilet for each of the households and cut down the practice of open defecation. Very recently, researchers have shown an estimate of 36% and 48% reduction in risk of having diarrhea by sanitation and hygiene interventions, respectively, in young children residing in Southeast Asia and African countries. Other than infections by the pathogens, a number of causes may also result into diarrheal symptoms e.g. malabsorption, lactose intolerance etc.

The main symptom of diarrhea is loose watery bowel motions three or more times a day while frequent passing of formed stool is not considered as diarrhea. Diarrhea has been categorized under three different groups which are i) acute diarrhea; lasting for several hours or days, ii) acute bloody diarrhea; sometimes called dysentery, and iii) persistent diarrhea which lasts for 14 days or longer.

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The disease is life threatening as it causes huge loss of fluid and electrolytes in the form of diarrhea. Mortality due to diarrheal cases are primarily due to severe dehydration. In fact, over the last few decades, diarrheal mortality has been reduced significantly due to use of oral rehydration solution to correct dehydration of the victim. In fact, discovery of ORS is considered as one of the best discoveries of the 20th century which saved millions of life with so little cost. Malnourished children and immunocompromised adults (like people living with HIV) are at the most risk from life threatening diarrhea.

Etiological agents of diarrhea:

Commensal organisms in the intestines provide most beneficial effect towards proper nutrition. In fact, even today, with the most advanced methods for culturing of the microorganisms we failed to culture over 50% of these resident organisms and these resident bacteria reside within a complex ecosystem that provides constant benefit to the host and the resident organisms. Advances in molecular based detection assays has given us opportunities for understanding structural and functional complexity of these commensals; it's a just beginning and our understandings are in a very infantile stage. Lumen of the intestine is covered with protective cover of mucus layer that provides initial physical barrier and is an important source of various types of nutrients, constantly providing growth support to commensals and sojourn of pathogenic organisms which arrive through the ingestion of contaminated food or water. To establish successful intestinal infection, pathogenic organisms multiply within the gut for effective colonization /invasion of intestinal mucosa and secrete various types of toxigenic factors to cause deleterious effects leading to outpouring of water into the lumen of the gut that comes out in the form of diarrhea.

Diarrhoeagenic pathogens are grouped into three main categories of organisms i) bacteria, ii) virus, and iii) parasites. Infection by these organisms are also commonly known as gastroenteritis. In reality, precise detection of the pathogen, diarrheal etiology, by conventional microbiological assays is cost intensive and is perhaps not necessary to perform for all cases of diarrhea. Reservation for detection of the enteropathogens may be made for patients in high risk group or for acute cases of diarrhea or in a situation when outbreak is evident like cholera outbreak. Comprehensive information on diarrheal etiologies are of definite help to assigning ranking of most common pathogens to cause the diseases and helping in setting up priorities and preventive measures to circumvent infections by these pathogens among the community

Hospitalized diarrhea cases at Kolkata showed that most of the patients had have acute diarrhea with various levels of dehydration. Prominent etiological agents that caused diarrhea were *Vibrio cholerae* O1, Rotavirus and *Giardia lamblia*. Association of Rota viral diarrhea was mostly among children below the age of 5 years. Other enteric viruses, *Shigella* spp. and different pathotypes of diarrheagenic *Escherichia coli* were also associated with hospitalized cases of diarrhea. It is normally believed that primarily one pathogen is to cause

“Antimicrobial resistance is not a future threat looming on the horizon. It is here, right now, and the consequences are devastating.”

Dr. Margaret Chan,
Director-General of the WHO



the disease. However, etiological assessment of the diarrheal stools from hospitalized cases revealed high degree of polymicrobial (more than one pathogen) infections. Surprisingly enough, about 30% of the stool specimens from acute diarrhea cases remain undiagnosed with no detection of potential pathogens. These could be cases of antibiotic usage prior to hospital admission or the pathogen counts remained below the detection limit of the conventional detection assays.

Culture independent detection of bacterial enteropathogens: are we seeing tip of the iceberg

Polymicrobial infections in diarrheal diseases are generally reported from resource poor countries and such kind of infections have been considered as a major factor contributing to severity of diarrhea. Despite using all modern microbiological assays, about 30% of the stool specimens from hospitalized acute diarrhea cases at Kolkata remain undiagnosed for etiological agents. Such situations have stressed the need for more sensitive, specific and rapid detection assays for identifying pathogens from diarrheal stool specimens. While culture based detection assays heavily rely on biochemical characterization of the pathogens that grew on selective plates, molecular detection is based on presence or absence of nucleic acid of the target pathogens in stool specimens. Getting pure culture and subsequent confirmation is still considered as gold standard but it takes considerable time to confirm the etiology. With the advancement of molecular assays, numerous culture independent rapid detection assays have been developed of which real-time PCR (RT-PCR) based assays have gained much interest. Attempts were made to detect presence of nucleic acid in diarrheal stools, particularly for those ~30% cases where detection of any pathogens remained obscure. Based on the existing surveillance data, identification of *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Campylobacter* spp., *Shigella* spp., and 3 different pathotypes of diarrheagenic *E. coli* was prioritized. A pool of specimens was selected where presence of single pathogen was known for 86.8% (n= 59) specimens by conventional culture based methods. Reanalysis by molecular methods indicated presence of nucleic acids from multiple pathogens. In fact, reanalysis of specimens where no known etiologies were identified



by the culture based methods, showed majority (63%, n= 34) of them contained nucleic acid of one or more pathogens. The prevalence of such high level of polymicrobial infections would not have been detected if RT-PCR was not utilized. It was possible to estimate relative pathogen load in these specimens which indicated the inability of conventional culture dependent methods to detect the pathogens was related to lower colony forming units of the pathogen. Interestingly, in many of the occasion, load of one pathogen was 100 folds or more than the other was detected. Detection of high levels of polymicrobial infection by RT-PCR indicated that in the settings like Kolkata and around, which is endemic for cholera and other enteric diseases, the concept of one pathogen one disease might need to be re-evaluated.

Concluding remarks

In low resource poor settings, polymicrobial infections in diarrheal cases are common and no exception among hospitalized acute diarrheal cases at Kolkata. This is in stark contrast to what is seen in developed country settings where the etiology of diarrhea is due to single pathogen. Not only existence of very high level of polymicrobial infections, the significance of contrasting densities (about 100 folds more of one pathogen as compared to another) of enteric pathogens in mixed infection scenario pointed out a need to have better understanding on complex nature of intra-microbial interactions. Apart from detecting pathogens, culture independent techniques for identifying and to characterize uncultivable normal floras are currently being perused in many laboratories. Such studies are the best way to understand their intricate interrelationship for human health.

Ranjan Kumar Nandy
Scientist E, Bacteriology Division

Library News: NICED library has received fund to the tune of 40 lakhs from ICMR in the year 2015-16. With this fund NICED library has added a good number of books and journals in its resource. These books are recommended by the scientists of the Institute, recommended and approved by the Library Committee and Director-in-charge

List of books purchased in 2015-16

1. Statistical aspects of community health and nutrition
2. Harrison's principles of internal medicine (2 vols)
3. Davidson's principles and practice of medicine
4. Park's textbook of preventive and social medicine
5. Bailey & Scott's diagnostic microbiology
6. Clinical biochemistry: metabolic and clinical aspects
7. Field's virology (2 vols)
8. Medical microbiology
9. Prescott's microbiology
10. Research methods for community change: a project based approach
11. Review of medical microbiology and immunology
12. Methods and applications of statistics in clinical trials: concepts, principles, trials and design (2 vols)
13. The water frame work directive: action programmes and adaptation to climate change
14. Nucleic acid biosensors for environmental pollution monitoring
15. Epidemiology: study design and data analysis
16. Chemiluminescence and Bioluminescence : past present and future
17. Modeling to inform infectious diseases control
18. Aquatic ecotoxicology: advancing tools for dealing with emerging risks
19. Analyzing and modeling spatial & temporal dynamics of infectious diseases
20. Protein dynamics: methods and protocols
21. Introductory statistics for the health sciences
22. Analysis of biomarker data: a practical guide
23. Bioinformatics & functional genomics
24. Quantitative microbial risk assessment
25. Biology of foodborn parasites
26. Mobile DNA III

27. Computer analysis of sequence data
28. Methods in molecular biology: nucleic acids
29. Practical protein chromatography
30. Methods in molecular biology: proteins
31. Protocols in molecular parasitology
32. Conn's current therapy
33. Roitt's essential immunology
34. Manson's tropical diseases
35. Mixed methods in health science research: a practical primer
36. Principles of protein X-Ray Crystallography
37. The human microbiota and microbiome
38. Sleisenger & Fordtran's gastrointestinal & liver diseases: pathophysiology/diagnosis/management (2 vols)
39. Oxford textbook of global public health (3 vols)
40. Current protocols in molecular biology (online)

List of Journals added in 2015-16

1. Nature Immunology
2. Nature Reviews Microbiology
3. Mucosal Immunology
4. Gastroenterology
5. Infection Genetics and Evolution
6. International Journal of Medical Microbiology
7. Journal of Clinical Epidemiology
8. Microbes and Infection
9. Protein Expression and Purification
10. Trends in Microbiology
11. Trends in Parasitology
12. FEMS Microbiology Reviews
13. FEMS Immunology & Medical Microbiology (now Pathogens and Diseases)
14. Cellular Microbiology
15. Antimicrobial Agents and Chemotherapy
16. Applied & Environmental Microbiology
17. Clinical & Vaccine Immunology
18. Clinical Microbiology Reviews
19. Eukaryotic Cell
20. Infection & Immunity
21. Journal of Bacteriology
22. Journal of Clinical Microbiology

23. Journal of Virology
24. Microbiology & Molecular Biology Reviews
25. Molecular & cellular Biology
26. Ecosal Plus
27. American Journal of Epidemiology
28. Journal of immunology
29. Vaccine
30. Journal of Medical microbiology
31. Epidemiology & Infection
32. Microbiology Spectrum

