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Connections

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Spreading the EQA word

About 10 years ago, CMPT started on a mission to help EQA programs around the world see the advantages of making their own stable, clinically relevant bacteriology samples as opposed to single function lyophilized ones.

IN THIS ISSUE

Working with organizations like the World Health Organization and interested governments, we found a substantial audience mainly in the Middle East, Asia, and southern Africa, and Europe.

At the time, the idea was to bring laboratorians to Canada rather than have us go to them. The thinking was sound, in large part because while we could do our own work and teach at the same time (thanks to CMPT staff, like Caleb and Esther); but equally important, there was no way that we could survive taking 10 day training trips and keep CMPT on schedule here. The program worked not too badly, with some of the visiting teams going back and having huge improvements.

But the program was not totally successful. Some of the programs sent the wrong people; managers rather than laboratory workers, and some went home and were not interested in follow-through. And for many, the cost of sending two people to Canada for two weeks was very, very, very expensive. It was not a sustainable project to find people interested from countries that could afford the training. And so the program stopped.

But by chance, the program had an opportunity to return, with an interesting partnership. While we were doing our onsite training CMPT developed a collaboration with another Canadian EQA program, that although may be weak in the area of making clinically relevant stabilized microbiology samples, is very strong in informatics and a range of other disciplines, some of which include fresh frozen serum. By changing our training program, we offered our visitors the opportunity to visit Oneworld Accuracy and learn about their informatics handling.

Following a lot of discussion we came to a new strategy. Rather than have people come here, through a different funding model through Oneworld Accuracy, I would be able to take the training abroad. I worked with their staff, especially their microbiologist, Jean Frederic Flandin, and we developed a related, but different training program which we just recently trialed in a laboratory near Abuja, Nigeria. (Of interest, Nigeria is almost the exact same size as British Columbia, but has over 200 million population and over 5,000 medical laboratories!)

Spreading the EQA word

Over a period of a week, we covered a lot of ground including stabilization of samples, homogeneity, and the creation of a number of different sample formats. While the training had a lot of challenges, on balance I felt we had some pretty substantial successes. We will continue to work with them at a distance, but with a goal that they will create samples for send-out before 2020.

As it turned out the training occurred in the same city where the African Society for Laboratory Medicine was hosting their annual meeting. People were met, discussions were held, and now plans are being made with several additional countries.

The bottom line truth is that laboratories around the world understand the value and importance of EQA as an important approach to monitoring laboratory Quality, and relying on mailed in samples from mega-organizations from developed countries is not an ideal (barely useful) go-forward strategy.

So we will see how things go.

Dr. M. Noble

NEWS

Legacy Data Entry System phased out

CMPT's old data entry system has been phased out.

The new system is now in use to enter data for all PT programs.

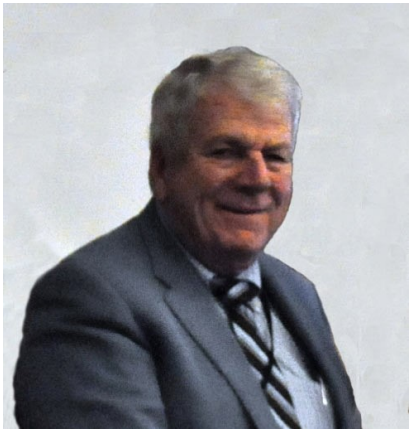
Laboratories will not have access to old reports. If you have any concerns please contact CMPT before February 28, 2019.

CMPT Professional Development course

The 2019 CMPT Professional Course has started.

Visit us at pd.cmpt.ca

The Polymyxin Complex – Colistin and Polymyxin B



Feature Article

By Dr. **Robert Rennie**

The polymyxin complex is part of a large complex of cyclic lipopeptides that include polymyxins and daptomycin. There are at least 15-16 of these molecules, but only the former are used in clinical medicine. They are subdivided into groups that affect gram-negative bacteria (polymyxin), gram positive bacteria (daptomycin), antifungal and anti-mycobacterial agents. Daptomycin is a semi-synthetic cyclic lipopeptide isolated from *Streptomyces roseoporus*. It has very different microbial and clinical activities than other polymyxins, a primary requirement of calcium ions for laboratory testing, and specific pharmacokinetics and pharmacodynamics.

This article will focus on the two major polymyxins (colistin and polymyxin B) that affect gram-negative bacteria. These compounds were originally isolated over 60 years ago from *Bacillus polymyxa*

Mode of action.

The polymyxins of note that affect gram-negative bacteria and are used therapeutically in most parts of the world are two related compounds – colistin (polymyxin E) and polymyxin B. They are large molecules with molecular weights between 1,150 and 1,200 daltons. They are water soluble except in alkaline solution. (between pH 5.5 to 7.0).

The polymyxins act by penetrating the cell-wall of several species of gram-negative bacteria. Because of their large molecular mass, they are able to penetrate the wall, by chelation of divalent cations, thus causing the cell wall to destabilize. They then interact in with components in the cell membrane (phosphatidylethanolamine) which is rich in gram-negative bacteria, but in low concentration in gram-positive bacteria. The precise mode of action is not fully understood, but it is likely that they act like a detergent within the membrane resulting in disruption of the membrane and leakage of intracellular moieties from the cell, thus killing the cell. Some investigators liken the action of the polymyxins to washing the cells in "soap".

Antimicrobial activity

It is somewhat difficult to measure the precise antibacterial activity of these compounds. They are not active against gram-positive bacteria. They are active against a variety of species of gram-negative bacteria, with MICs that vary from 0.01 mg/L to 8 – 16 mg/L. It appears that *Proteus* species, and *Serratia marcescens* are intrinsically resistant (MICs > 128 mg/L). Broadly, some *Enterobacteriaceae* (*Escerucgua coli, Klebsiella, Enterobacter, Salmonella, Shigella*) and *Pseudomonas aeruginosa* are affected by both polymyxin B and colistin, whereas only limited data is available on other genera for colistin (*Yersinia, Acinetobacter, and Citrobacter*).

In vitro laboratory testing

Most testing in the laboratory is performed using colistin and both microdilution with cation-adjusted Mueller Hinton broth. It is important to use colistin sulfate in the test, because colistinmethanesulfonate is a pro-drug of colistin, and conversion to the active compound may cause testing issues and errors in MIC tests. Because the polymyxins are such large molecules, testing needs to be standardized as much as possible. Broth microdilution trays should be made in plain untreated polystyrene. Additives such as polysorbate 80 are not recommended.

Susceptibility testing by other methods such as disc diffusion, gradient-endpoint diffusion and agar dilution are not recommended. The antimicrobials diffuse variably from discs, and the large molecular weight of the agents does not lend well to methods other than broth-micro-dilution. Studies on other methods continue to be investigated, but until the obstacles mentioned can be overcome they must remain in the research environment.

Quality control ranges for broth micro-dilution have been established by EUCAST and CLSI for E. coli ATCC 25922 (0.25 – 2.0 mg/L) and *P. aeruginosa* (0.5 – 4.0 mg/L). There are no clinical breakpoints established in EUCAST – only Epidemiological Cut off Concentrations (ECOFFs). For some *Enterobacteriaceae* (eg E. coli, Enterobacter cloacae, Klebsiella) the ECOFF is 2.0 mg/L; for *P. aeruginosa* the ECOFF is 4.0 mg/L. There are no ECOFFs for *Acinetobacter baumannii* complex. CLSI, in it's M100:2019 version reports breakpoints of ≤ 2.0 mg/L (S), and ≥4.0 mg/L (R) for *P. aeruginosa* and *A. baumannii* complex , and ECOFFs of ≤ 2.0 mg/L for *Klebsiella, E. coli, Enterobacter cloacae* and *Raoultella*.

CLSI also provides disc diffusion quality control ranges of 11 – 17 mm for *E. coli* ATCC 25992 and *P. aeruginosa* ATCC 27853. There are no disc diffusion quality control ranges in current EUCAST documents. Until there is better understanding of the linkage between the pharmacokinetics of colistin and appropriate breakpoints it is recommended that only the ECOFFs are used to tentatively determine if an isolate is more likely to be relatively susceptible to the therapeutic polymyxins. Similarly disc quality control ranges should only be considered tentative until there is consensus from international groups.

KEY LEARNING POINTS

- Polymyxin B and colistin are effective on gram-negative bacteria only
- These agents have specific requirements for *in vitro* testing
- There are no clear clinical interpretation breakpoints
- Usually used in combination therapy

Polymyxin resistance

Antimicrobial resistance to the polymyxins has historically been relatively uncommon. Reports have been made regarding resistance in *P. aeruginosa, A. baumannii* complex and *K. pneumoniae*, but these are uncommon. Recently, it has been shown in several studies that colistin resistance can be plasmid-mediated, and this has raised concern about clinical use of the drug in mixed population infections.

Further, it has also been shown that hetero-resistance to colistin may occur in *P. aeruginosa* and *A. baumannii* complex isolates. Despite rapid bactericidal effects of the agent, re-growth has been observed in the presence of high concentrations of the drug, suggesting that small proportions of the population in these isolates are highly resistance to colistin and polymyxin B.

Pharmacokinetics/Pharmacodynamics/Toxicity

Almost all studies indicate that the polymyxins are excreted by non-renal pathways.

Studies with multi-drug resistant *P. aeruginosa, Klebsiella* and *A. baumannii* complex indicate that the polymyxins have potent concentration dependent killing of the species. There in no known post-antibiotic effect.

In studies on the plasma concentrations of active colistin, it has been observed that the active agent (colistin) reaches concentrations of between 0.5 and 3 mg/L 6 – 8 hr after administration. The peak concentrations are not significantly affected by renal function suggesting there is re-absorption from the kidneys and further that renal excretion is insignificant.

The pro-drug (colistinmethanesulfonate) is excreted through the kidneys. There is further data regarding the conversion to active colistin that suggests clinically two important factors. 1) The important PK/PD parameter for the polymyxins is the Area under Curve/ MIC (AUC/MIC), and 2) the peak concentrations are not significantly greater than MICs that would support use of the polymyxins alone for treatment despite their apparent potent concentrations dependent killing of bacterial cells.

The most common adverse reactions and toxicity have been described as nephro- and neuro-toxicities. Allergic reactions may also occur, but the frequency is relatively smaller. Colistin has been used in the cystic fibrosis population with relatively few side effects, in cystic fibrosis patients and in cases of bronchiectasis, but usually in combination with other agents or surfactants.

Summary

The foregoing indicates that the therapeutically active polymyxins (colistin and Polymyxin B) have a relatively narrower spectrum of activity. but may be effective if used in combination with other susceptible antimicrobial agents. In susceptible gram-negative species they have rapid concentration dependent killing by intercalation with cations (calcium and magnesium) in the bacterial cell membrane and disruption of the membrane in a detergent-like process. Leakage of internal cell contents causes rapid killing.

The relative lack of resistance is potentially a positive attribute of these agents, but they do not reach high AUC/MIC ratios such that their use alone for effective treatment is suspect.

Antimicrobial susceptibility testing is still problematic and requires more extensive study to establish clinical breakpoints and quality control parameters for other that broth-microdilution tests.

The current problematic issue is routine testing in the clinical laboratory. It is really only currently possible to accurately perform broth micro-dilution testing according to parameters described above. With quality control in place, isolates of appropriate species that have MICs < 2 mg/L may be considered relatively susceptible. In those cases, colistin may be useful if used in combination with another agent to which the micro-organism is susceptible. Most laboratories at this time will be unable to confidently perform testing and reporting, so isolates for test will need to be sent to reference laboratories that are equipped to undertake such investigations under prescribed conditions.

Dr. Rennie is a Professor Emeritus (University of Alberta) and Clinical Microbiology Consultant, member of the Canadian Standards Association ISO Z252/TC212 Committee on Quality Laboratory Management, Deputy Convener of ISO TC212 Working Group 4 (Microbiology and Molecular Microbiology), and member (former Chair) of the Clinical Microbiology Proficiency Testing (CMPT) Microbiology Subcommittee.

Some aspects of this article reflect opinions of the author based on current understanding of the topic.

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Welcome Dr. Kathy Malejczyk to the Clinical Bacteriology committee

We would like to give Dr. Malejczyk a very warm welcome to CMPT's Clinical Bacteriology committee of experts. Dr. Malejczyk is a medical microbiologist at Regina Qu'Appelle Health Region.

Upcoming Events

APRIL 2019

2019 AMMI Canada – CACMID Annual Conference

April 3-6 Ottawa, ON
More info: http://www.cacmid.ca/2018/09/2019_ottawa/

29th ECCMID

April 13-16 Amsterdam, Netherlands
More info: http://www.eccmid.org/

MAY 2019

World Conference on Quality Improvement

May 20-22, Fort Worth, TX
More info: https://asq.org/conferences/wcqi

LABCON 2019

May 24-26, Fredericton, NB
More info: https://labcon.csmls.org/

ABOUT CONNECTIONS

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