SUPPLEMENTARY MATERIAL

Title

Antibiotic activity of the antioxidant drink effective Microorganism-X (EM-X) extracts against common nosocomial pathogens: an in vitro study.

Authors

Alessio Mancini[1], Sandra Pucciarelli[1]

Affiliations

1. *School of Biosciences and Veterinary Medicine, University of Camerino, 62032, Camerino Italy*

Abstract

Nosocomial infections are one of the leading causes of mortality in hospitalized patients. The desire for new products offering better tolerance by patients with less toxicity has led to an increase in research on natural products. EM-X is a mixed consortium of beneficial microorganisms of natural occurrence (lactic bacteria, yeast and photosynthetic bacteria). The aim of this study is to evaluate the antimicrobial activity in-vitro of EM-X to the principal pathogens isolated in clinical settings and to understand if it could be a suitable adjuvant to synthetic antibiotics. According the *World Health Organization* we performed antimicrobial assays to the main pathogens usually found in hospital wards. After antimicrobial testing, EM-X has been shown to be most effective at a concentration of 40 mg/ml four time concentrated than the commercial original solution (10 mg/ml). The EM-X antimicrobial action, although effective against bacteria, has proved to be ineffective against the *candida* genus.

Keywords

EM-X, Effective Microorganisms, antibiotic activity, flavonoids, nosocomial bacteria

Experimental section

The EM-X solution (Lot.AG10015) was obtained from local traders. The liquid inside the bottles from three different production batches has been mixed to reduce the risk of a defective product. This EM was based on the original formulation developed by Higa and Parr known as EM-1(Higa, T., & Parr 1994). According to standard EM inoculation procedure, its activation was carried out by incubation at 35 C for 7 days and microbial activity was detected through changes in pH and sugar concentration. The juice was centrifuged, the pellet was discharged, and the surnatant was

lyophilized to dryness to obtain a powder extract. The dried extract was then dissolved in sterile distilled water to obtain a concentration of 80 mg/ml. The concentrated EM-X was further filtered by a 0.22-µm filter and the filtrate was then stored in aliquots at -20˚C until use.

The strains of this work have been isolated in a tertiary care hospital in center of Italy. The main pathogens usually found in hospital wards has been used (WHO 2014): among Gram+ bacteria *Staphylococcus aureus* and *epidermidis, Streptococcus agalactie* and *Enterococcus faecalis*; among Gram- bacteria *Serratia marcescens, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumanii, Escherichia coli, Citrobacter koseri, Pseudomonas aeruginosa;* among fungi *Candida Parapsilosis, albicans, tropicalis, krusei and glabrata.* All identifications and antimicrobial susceptibility tests were performed with VITEK®2 (Bio-Mérieux, Marcy l'Etoile, France*)* and the microorganisms had neither particular resistances nor MDR (Multiple drug resistance) in order to represent the most common pool in hospitals. Pure cultures of all experimental bacteria and fungi were from the American Type Culture Collection (ATCC) and obtained from the University of Camerino (Italy). The MIC (Minimum Inhibitory Concentration) for every microorganism was defined using sterile 96-well microdilution plates and each test was repeated twice. Inoculum was prepared according to CLSI direct colony suspension method (Clinical and Laboratory Standards Institute 2013). Cultures for experiments were prepared by transferring a loopfull of cells from the stock cultures to test tubes of nutrient broth (Mueller-Hintthon broth, Oxoid, UK) and incubated for 24 h at 37°C to achieve a turbidity of 0.5 McFarland. The suspensions were diluted 1:10 in fresh Mueller-Hinton broth to obtain a concentration of 10^7 CFU/ml and 2 µl were inoculated to each microplate well. Every sample concentration was checked by a spectrophotometer (VWR Collection, UK). The assay was performed inoculating the EM-X extract to achieve a final microorganism density of approximately 10^4 CFU per spot and a column was used as the blank. When satisfactory growth was obtained (18-36 hours) the plates were scanned with an ELISA reader and result were reported in tables.

Bibliography

Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement.

Higa, T., & Parr JF. 1994. Beneficial and effective microorganisms for a sustainable agriculture and environment. Int Nat Farming Res Cent. 1:1–16.

WHO. 2014. ANTIMICROBIAL RESISTANCE: Global Report on surveillance. World Heal Organ. doi:10.1017/CBO9781107415324.004.