Antimicrobial effect of OM-X against Clostridium difficile

What is Clostridium difficile?

Clostridium difficile (C.difficile) is a species belonging to the genus Clostridium known as one of the causative agents of food intoxication. C.difficile is a kind of bacteria that always stays in the colon (indigenous bacteria) which usually does not produce toxins. However, since this strain is a bacterium to which many kinds of antibiotics are not effective (resistant strain), when other intestinal bacteria are killed off by application of antibiotics, only this strain survives, proliferates abnormally (microbial substitution), and begins to produce toxins. These toxins (enterotoxin and cytotoxin) produced cause disorders to the mucous membranes of the intestinal tracts where in the mild case, patients have loose stool; and in the serious case, these trigger pseudomembranous colitis (PMC) that patients suffer from severe diarrhea, stomachache and high fever. With the development and common use of various kinds of antibiotics since the second half of the twentieth century, the number of patients suffering from antibiotics-related diarrhea is also increasing. Diarrhea by C.difficile has continued to be acquired nosocomially in patients and health care personnel to the present day. Nowadays, people are concerned about the increasing number of people infected after the use of antibiotics prescribed in general as well. In order to treat this disease, stopping intake of antibiotics for a while and taking vancomycin or metronidazole are commonly known. Meanwhile another treatment option is also reported that C.difficile diarrhea was suppressed by the probiotic effect of lactic acid bacteria (Reference: Am J Gastroenterol. 2000 Jan; 95(1 Suppl):S11-3). The most important ways of prevention can be described as not to break the balance of intestinal flora with overuse of antibiotics for non-specific problems and to keep the intestinal flora in a favorable state.

Antimicrobial test of OM-X against C.difficile

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OBJECT

To investigate the antimicrobial activity of OM-X (contents of the capsule) against Clostridium difficile (JCM1296).

METHODS

Culture of test strain

After Clostridium difficile (JCM1296) was cultured in GAM bouillon (Nissui Pharmaceutical Co., Ltd., Tokyo) for 18 hours, culture solution with the bacterial number approximately 9×10^{8}/milliliter (mL) was gained. The culture condition was at 37℃ in an anaerobic culture.

Preparation of test medium

One hundred microliters of the above cultured solution of C.difficile was added to 10mL of GAM

culture medium with 0.7% agar (approximately 9×10^6/mL as the final bacterial concentration). Then it was poured to a petri dish to make a soft agar plate. After the agar solidified, a well of 9mm in diameter was produced in the center of the plate to use this plate as a test medium.

Addition and culturing of the test solution

The contents of OM-X capsule was diluted with sterilized distilled water in the ratio of 8 to 2 to make the test solution.

Agar well method

Eighty microliters of the test solution in which OM-X was diluted was added to the well in the test medium and left statically at 16℃ for 2 hours. Then, this is test medium was cultured at 37℃ for 24 hours in an anaerobic condition. After incubation, the presence or absence of growth inhibitory zone for the bacteria around the well was confirmed. In the meantime, a test medium was cultured as a negative control in which no test solution was added to the well of the test medium.

RESULTS

A bacterial growth inhibitory zone with a diameter of 20mm was confirmed around the well situated in the center of the test medium (Fig. 1). In the test with the negative control, no bacterial growth inhibitory zone was observed (Fig. 2). The brown color tone found around the well in Fig. 1 is the tone of OM-X itself.

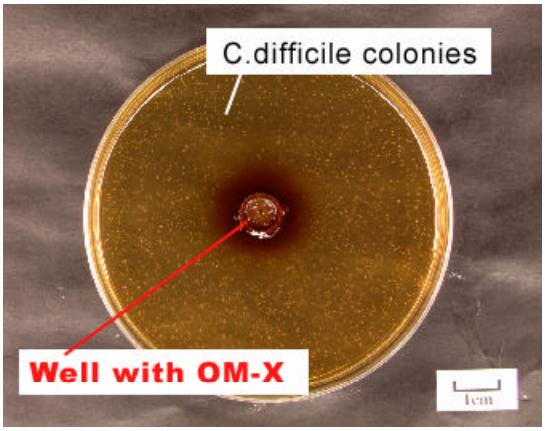

Fig. 1 Antimicrobial activity of OM-X against C.difficile
The hole (well) at the center of the petri dish is filled with the solution in which the contents of OM-X was diluted. In the area with a diameter of 20mm around the well (area indicated with a blue arrow), C.difficile colonies (white dots) did not proliferate. The part seemed brown is the tone of OM-X itself.

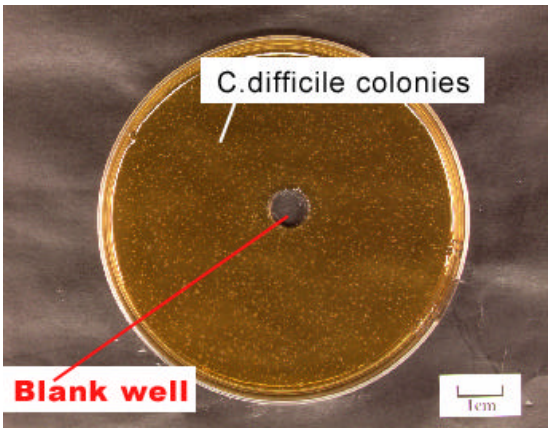

Fig. 2 Test with C.difficile -negative control
The hole (well) at the center of the petri dish is left unfilled. C.difficile colonies (white dots) proliferate entirely.