JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2006, p. 378–382 Vol. 44, No. 2
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.2.378–382.2006

Influence of Anti-FloR Antibody on Florfenicol Accumulation in Florfenicol-Resistant *Escherichia coli* and Enzyme-Linked Immunosorbent Assay for Detection of Florfenicol-Resistant *E. coli* Isolates

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Received 2 May 2005/Returned for modification 15 June 2005/Accepted 16 November 2005

To detect florfenicol-resistant *Escherichia coli* isolates by enzyme-linked immunosorbent assay (ELISA), anti-FloR1 antibodies were produced in mice using a recombinant glutathione *S*-transferase (GST)-FloR1 protein, which was expressed in a prokaryote expression system, as the antigen. The specificity of the murine anti-GST-FloR1 antibody and its influence on florfenicol accumulation in florfenicol-resistant isolates were investigated using Western blotting and high-performance liquid chromatography, respectively. Western blotting using the anti-FloR1 antibody showed specific binding of the antibody to the florfenicol-resistant FloR protein. Preincubation of florfenicol-resistant strains with the antibody significantly increased the intracellular accumulation of florfenicol and enhanced the bacterial susceptibility to florfenicol, suggesting that antibody binding to the FloR protein inhibited the activity of the efflux protein conferred by the *floR* gene. Analyses of florfenicol-resistant and -sensitive isolates by ELISA using the anti-FloR1 antibody showed good correlation between FloR protein expression and the *floR* genotype. The anti-FloR1 antibody-based ELISA is a useful tool for the detection of florfenicol-resistant bacteria harboring the *floR* gene.

Florfenicol, a novel broad-spectrum antibiotic, is a fluorinated analog of thiamphenicol and chloramphenicol. It has not been approved for use in humans. From the 1990s, it has been used in many countries for the treatment of bacterial infections in animals such as cattle, pigs, poultry, and fish. The widespread use of florfenicol has resulted in the development of cross-resistant bacterial pathogens that may enter the food chain and potentially result in food-borne illnesses in humans (24). The florfenicol resistance gene was first detected in 1996 from a fish pathogen, *Pasteurella piscida* (19), and the gene was later identified in a chromosomal multiresistance gene cluster of the definitive *Salmonella enterica* serovar Typhimurium phage type DT104 (2, 3, 8, 10, 18). This antibiotic resistance gene cluster of about 13 kb is located in a chromosomal genomic island called *Salmonella* genomic island 1 (SGI1). SGI1 or variants of SGI1 have also been identified at the same chromosomal location in another *S. enterica* serovar, Agona (9, 13). The resistant gene was also identified in plasmids and the chromatin of *E. coli* (4, 6, 7, 12, 14, 17, 24), in the IncC plasmid R55 from *Klebsiella pneumoniae* (11), and in *Vibrio cholerae* (16). These studies showed that the genes, referred to in the published literature as *pp-flo*, *cmlA*-like, *floSt*, *flo*, or *floR*, mediate combined resistance to chloramphenicol and florfenicol. Despite the different designations, these genes are closely related and show 96 to 100% identity in their nucleotide sequences (22), and the resistance gene (hereafter referred to as *floR*) could disseminate via a high-molecular-weight plasmid and/or a putative mobile transposon (24).

This study was initiated to develop a rapid enzyme-linked immunosorbent assay (ELISA) to detect bacteria that carry the *floR* gene and thus monitor the developing trend of florfenicol resistance. For the ELISA, a murine antibody against the protein expressed by the *floR* gene was produced following the production of a recombinant protein (referred to as FloR1) in *E. coli*.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used in the experiment are listed in Table 1. Nine *E. coli* strains (C83xxx series) were isolated from calf diarrhea cases and identified by China Agricultural University and the China Institute of Veterinary Drug Control. The resistant strain CVM1841 was kindly donated by David G. White from the FDA and has been previously described (24). The resistant strain JM109-R and the florfenicol-sensitive control strain pGEM-T/JM109 were constructed from JM109 in our laboratory (14). *E. coli* strain BL21-codon plus (DE3)-RP (named CP-RP) used for FloR protein expression was kindly donated by the Department of Microbiology and Immunology, China Agricultural University. The bacterial strains were stored at −86°C before use.

Construction of the FloR1-expressing system. A prokaryote expression system was used to express the FloR1 protein. In brief, two primers, one upstream primer (flo 1, 5′-GCGATGGGATCCCTC CTAAATGCGGGTTTC-3′) and one downstream primer (flo 2, 5′-CGCGACGAATTCGAAGGCAAAGCTGA ATCC-3′), were designed using the Oligo6.0 software based on the published *floR* gene sequence (GenBank accession no. AF231986). The plasmid DNA was extracted from *E. coli* CVM1841 using the Wizard Plus SV Minipreps DNA purification kit (Promega) and was used as a template DNA for PCR. The cycling condition of PCR included an initial denaturation at 96°C for 5 min, followed by 32 cycles of 94°C for 50 s, 58°C for 20 s, 72°C for 25 s, and 72°C for 10 min.

The PCR product was digested with BamHI and EcoRI and ligated to the vector pGEX-4T-2 (Amersham Pharmacia Biotech) to generate plasmid pGEX4T-*floR*, which was subsequently transformed in CP-RP cells. The plasmid pGEX4T-*floR* in a positive clone which could replicate in LB agar in the presence of 100 μg ml^{-1} ampicillin was sequenced. The recombinant strain was

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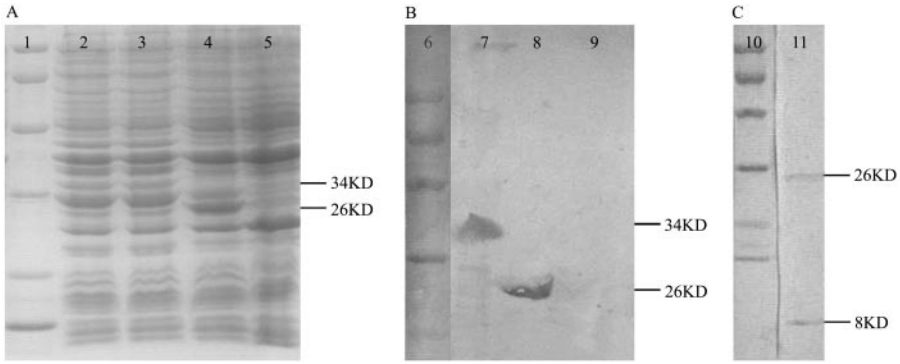

FIG. 1. Identification of the recombinant FloR1 protein. Lanes 1, 6, and 10 contain marker proteins of 97.4, 66.2, 43.0, 31.0, 20.1, and 14.4 kDa. (A) Coomassie blue-stained gel of SDS-PAGE of proteins from whole cells. Lanes 2 and 3, CP-RP/pGEX-216; lane 4, CP-RP/pGEX-4T-2; lane 5, CP-RP. (B) Western blot with anti-GST antibody. Lane 7, CP-RP/pGEX-216; lane 8, CP-RP/pGEX-4T-2; lane 9, CP-RP. (C) Western blot with anti-GST-FloR1 antibody. Lane 11, purified GST-FloR1 fusion protein was digested by thrombin and subjected to immunoblotting with anti-GST-FloR1 antibody.

named CP-RP/pGEX-216. The vector pGEX-4T-2 without the *floR* gene was also transformed in CP-RP cells, which were used as negative controls (CP-RP/pGEX-4T-2).

Expression and identification of the recombinant FloR1 protein. A large-scale (1-liter) CP-RP/pGEX-216 culture was incubated at 37°C. When the culture reached a turbidity reading at an A_{600} of 0.6 to 0.8, 100 mM IPTG (isopropyl-β-D-thiogalactopyranoside) was added at a final concentration of 0.1 mM to induce the expression of the fusion protein (glutathione *S*-transferase [GST]–FloR1). The culture was incubated at 30°C for another 4 h. CP-RP and CP-RP/pGEX-4T-2 were cultured under the same conditions as controls.

The bacterial cultures were centrifuged at 5,000 rpm for 10 min and resuspended in ice-cold phosphate-buffered saline (PBS). Bacteria were lysed by the sonication and freeze-thaw method (two freeze-thaw cycles). The GST-FloR1 fusion protein was identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting using an anti-GST antibody according to the manufacturer's instructions (Amersham Biosiences). The fusion protein GST-FloR1 was purified using the bulk GST purification module (Amersham Biosciences) according to the manufacturer's instructions.

Production of anti-FloR1 antibodies. Female BALB/c mice were used to produce anti-FloR1 antibodies by using a method modified by Lin et al. (20). Each mouse was injected with 100 μg of purified GST-FloR1 with complete Freund's adjuvant at the ratio of 1:1 for the primary immunization and then with five booster doses of GST-FloR1 (100 μg) and incomplete Freund's adjuvant (1:1) at 2-week intervals. Antisera were obtained 7 days after the last booster dose.

The antibody raised against GST-FloR1 was a polyclonal antibody. Binding of the antibody to both GST and FloR1 proteins was determined. The purified fusion protein was digested with thrombin according to the manufacturer's instructions supplied with the bulk GST purification module (Amersham Biosiences). The samples were subjected to Western blotting using the antisera diluted to 1:2,000 and goat-anti-mouse immunoglobulin G (IgG)-horseradish peroxidase (HRP), with incubation periods of 45 min (at room temperature) after the addition of each antibody.

Western blot assay of the *E. coli* isolates. The binding specificity of the antibody to FloR protein was confirmed by immunoblotting using the membrane fraction of florfenicol-resistant *E. coli* strains (JM109-R and CVM1841) and the florfenicol-sensitive (negative-control) strains (pGEM-T/JM109). The bacterial isolates were separately incubated in LB medium with florfenicol (final concentration, 32 μg ml^{-1}) overnight to induce the expression of FloR protein. After incubation, bacteria were harvested by centrifugation and then resuspended in 100 mM Tris-HCl buffer containing 20% (wt/vol) sucrose and 10 mM Na_3EDTA. A lysozyme solution (5 mg ml^{-1}, freshly prepared) was added to the bacterial suspension, and the mixture was incubated on ice for 10 min. After centrifugation at 4,500 rpm for 10 min, the pellet was washed using the same buffer and resuspended in 100 mM Tris-HCl containing 20% (wt/vol) sucrose, 10 mM $MgCl_2$, and 50 μg ml^{-1} DNase. Bacteria were lysed using the sonication and freeze-thaw method and centrifuged at 4,500 rpm for 5 min, and the supernatant was centrifuged at 100,000 × *g* for 20 min to yield a cytoplasmic (supernatant) and a membrane (pellet) fraction (1, 5). Proteins in both fractions were precipitated with 5% trichloroacetic acid. The precipitate was washed in acetone and then resuspended in 2× SDS loading buffer. All samples were subjected to electrophoresis by SDS-PAGE and transferred to a nitrocellulose membrane (15) for Western blotting with the antisera. After confirmation of the specificity of the antibody, the presence or absence of the FloR protein expressed by the *floR* gene in other *E. coli* isolates was determined using the same method.

Effects of the antibody on bacterial florfenicol accumulation. Assays of the intracellular accumulation of florfenicol were performed as previously described with some modifications (14, 23). Briefly, *E. coli* isolates were cultured in NB medium (0.1% tryptone, 0.2% yeast extract, 2% nutrient broth, 0.5% glucose) overnight. The culture was then diluted 100-fold in fresh NB medium and grown to an A_{600} of 0.6 before 6 mg ml^{-1} lysozyme and 0.01 mmol liter^{-1} EDTA were added. The incubation was continued for another 15 min before cells were harvested by centrifugation. The bacterial pellet was resuspended in SMM buffer (0.5 M glucose, 0.02 M $MgCl_2$, 0.02 M maleic acid, pH 6.5) to a final bacterial concentration of 40 mg ml^{-1}. The mouse antisera were added to the bacterial suspension at the final dilution of 1:10,000, and the culture was incubated at 37°C for 10 min before the addition of florfenicol (2 μg ml^{-1}). The same cultures incubated without the antisera were used as controls.

Two-milliliter samples were removed at 5-min intervals. The samples were immediately placed on ice and then centrifuged at 12,000 rpm at 4°C for 1 min. The pellet was washed once with ice-cold PBS buffer, resuspended in 2 ml of 0.1 M glycine hydrochloride (pH 3.0), and stored at 4°C for at least 15 h for full lysis. The samples were then centrifuged at 13,000 rpm for 10 min. The florfenicol concentration in the supernatant was measured by high-performance liquid chromatography (HPLC). The extraction procedure and HPLC method used for the determination of florfenicol have been previously reported (14). The results were expressed as nanograms of florfenicol incorporated per milligram (wet weight) of bacteria. The experiments were performed three times to confirm reproducibility, and the results were represented by means ± standard deviations (SD).

Effects of the antibody on bacterial susceptibility to florfenicol. Each isolate was cultured with broth medium at 37°C for 16 h. The susceptibility to florfenicol of all isolates incubated with or without the antisera was determined by broth microdilution according to the methods described by the CLSI (formerly the NCCLS) (21).

ELISA. (i) Assay development. The optimal dilution of the anti-FloR1 antisera was determined by ELISA. Antibodies with nonspecific binding to *E. coli* were removed by preincubation of the antisera with JM109 at the ratio of 10,000:1 (i.e., 1 ml antisera plus 0.1 μl JM109 at an A_{600} of 0.6) at 37°C for 30 min and centrifugation at 13,000 rpm for 1 min before use. The ELISA was performed as follows.

A polystyrene microtiter plate was coated with 75, 150, 300, or 600 ng/ml (in 100 μl) purified GST-FloR1 protein and incubated at 37°C for 1.5 h. After being washed with the washing buffer (PBS-Tween 20 [PBS-T]) three times, each well of the plate was filled with 100 μl blocking buffer (1% bovine serum albumin in PBS) and incubated at 37°C for 1 h. The antisera (or serum from an unimmunized mouse) in a series of dilutions from 1:200 to 1:25,600 in PBS-T buffer was added to the microtiter plate and incubated at 37°C for 1 h. The plate was washed again, and then to each well was added 100 μl 1:5,000-diluted goat-anti-mouse IgG-HRP conjugate in PBS and incubated at 37°C for 1 h. The plate was filled with tetramethyl benzidine containing 4 mM H_2O_2 and incubated for 15 min at

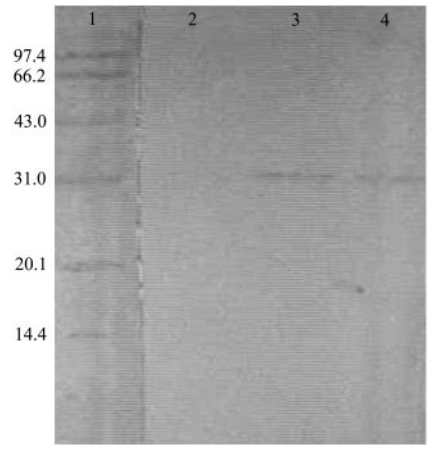

FIG. 2. Western blot of the membrane preparation of *E. coli* isolates. Lane 1, marker proteins of 97.4, 66.2, 43.0, 31.0, 20.1, and 14.4 kDa; lane 2, pGEM-T/JM109; lane 3, JM109-R; lane 4, CVM1841.

37°C for color development. The enzyme reaction was stopped by adding 50 μl 0.1 M citric acid, and absorbance was measured at 450 nm using a microplate reader (TECAN/A-5082).

(ii) Measurement of FloR protein concentrations in bacterial isolates. FloR protein concentrations in *E. coli* isolates were determined by the above-described indirect competitive ELISA using 300 ng/ml coating antigen. A bacterial suspension at an A_{600} of 0.6 was lysed by sonication and diluted to 1:1,000 in PBS-T. A 1:1 mixture of the diluted bacterial sonicate and 1:3,200-diluted antisera was added to the GST-FloR1-coated microtiter plate (final antibody dilution, 1:6,400). The plate was incubated, washed, incubated again with the goat-anti-mouse IgG-HRP conjugate and tetramethyl benzidine, and read as described above.

Purified FloR1 protein was used to construct the standard curve. The FloR1 protein (0, 6.25, 12.5, 25, 50, 100, and 200 ng/ml) was prepared in JM109 sonicate diluted 1:1,000 with PBS-T (A_{600} = 0.6) and then mixed with the antisera. The rest of the ELISA procedure was the same as that described above.

RESULTS AND DISCUSSION

Expression of the recombinant FloR1 protein. The *floR* gene product was previously predicted to be a 404-amino-acid protein of ca. 43 kDa (2, 24). Hydropathy analysis of the *floR* gene product revealed 12 hydrophobic putative transmembrane domains (2, 22). The amino acid region from sites 183 to 254 seemed the largest hydrophilic segment containing antigen determinants. Therefore, the segment (216 bp) of the *floR* gene predicted to be a protein (FloR) of 8 kDa was cloned into the pGEX-4T-2 vector and transformed into the CP-RP competent cells.

The GST-FloR1 fusion protein was successfully expressed using the prokaryote expression system. SDS-PAGE and Western blotting with anti-GST antibody of the whole-cell preparation of the recombinant strain pGEX-216/CP-RP showed a prominent band of 34 kDa, which was not observed for the FloR1-negative strains CP-RP/pGEX-4T-2 and nontransformed CP-RP (Fig. 1A and B). The 34-kDa protein was consistent with the prediction by the DNASTAR software and was the GST-FloR1 fusion protein. The control strain CP-RP/pGEX-4T-2 had a prominent band at 26 kDa of the GST protein (Fig. 1B).

Anti-GST-FloR1 antibody. Anti-GST-FloR1 antibodies were produced in BALB/c mice following injection of the purified GST-FloR1 fusion protein with Freund's adjuvant. Several booster doses were necessary to induce maximal antibody production. Western blotting with anti-GST-FloR1 antibody of the purified GST-FloR1 fusion protein, which was digested by thrombin, showed that the antibody bound to both the 26-kDa GST protein and 8-kDa FloR1 fragment (Fig. 1C).

Western blot assay of the *E. coli* isolates. Western blotting was used to confirm binding of the antibody with FloR protein encoded by the *floR* gene (Fig. 2). Immunoblotting using the membrane preparation of the known florfenicol-resistant strains carrying the *floR* gene (CVM1841 and JM109-R) (14, 24) and the mouse antisera displayed a protein band with a molecular mass of 30 kDa for both strains. In contrast, there were no positive bands in the membrane preparation of pGEM-T/JM109, which does not carry the *floR* gene. The molecular mass of this protein differs from the previously predicted value of 43 kDa (2, 24). It is possible that the signal peptide of the FloR protein was removed during synthesis within bacteria to yield a mature-form protein of 30 kDa. The Western blot results showed that the antibody had specific binding to the FloR protein.

No protein bands were detected for the cytoplasmic fraction by Western blotting using the antisera. This was consistent with previous findings that the florfenicol-resistant FloR protein was a membrane protein (2) which was classified within the

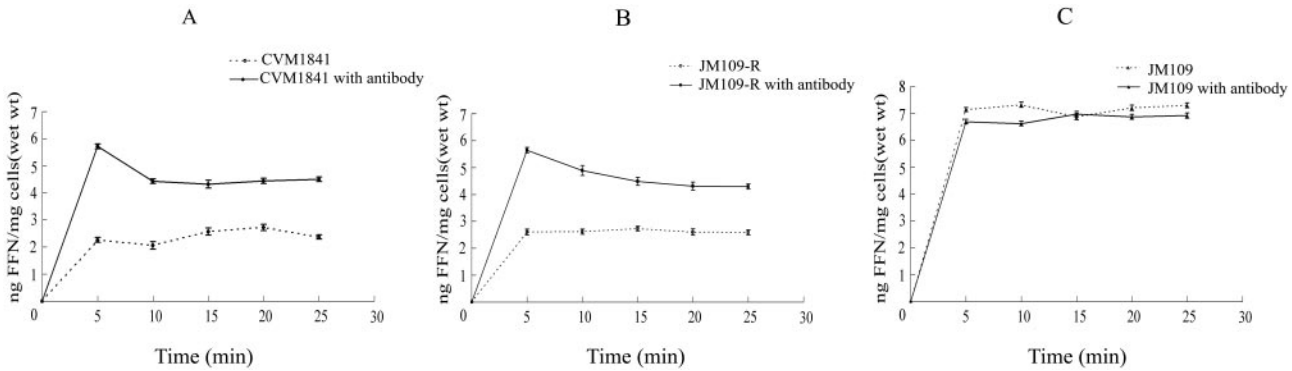

FIG. 3. Effect of anti-FloR antisera on florfenicol accumulation in *E. coli*. Incubation of bacterial isolates with the antisera significantly increased the intracellular accumulation of florfenicol in resistant strains (CVM1841 and JM109-R) but not in the florfenicol-sensitive strain (JM109). FFN, florfenicol. Values are means of results with three HPLC samples ± SD.

TABLE 1. Effect of anti-FloR antibody on bacterial susceptibility to florfenicol and the detection of FloR protein by ELISA

E. coli strain	MIC (μg · ml^{-1})		*floR* gene	ELISA OD[a]	FloR1 concn (ng/ml)
	Untreated	Antibody treated			
JM109	2	2	−	1.147 ± 0.009	1.053 ± 0.058
T/JM109	2	2	−	1.119 ± 0.041	1.238 ± 0.324
CVM1841	>128	32	+	0.330 ± 0.058	113.983 ± 45.381
JM109-R	64	32	+	0.419 ± 0.027	68.424 ± 11.620
C83260	128	64	+	0.287 ± 0.048	145.854 ± 45.762
C83261	2	2	−	1.166 ± 0.013	0.946 ± 0.075
C83262	32	16	+	0.641 ± 0.046	19.196 ± 5.804
C83286	32	8	+	0.557 ± 0.052	31.133 ± 10.713
C83287	2	2	−	1.100 ± 0.066	1.348 ± 0.635
C83302	4	4	−	0.973 ± 0.028	2.866 ± 0.496
C83305	2	2	−	1.147 ± 0.034	1.055 ± 0.131
C83914	128	32	+	0.433 ± 0.020	63.388 ± 7.718
C83916	64	32	+	0.499 ± 0.040	43.416 ± 11.088

[a] Absorbance at 450 nm. The mean OD reading and SD of positive antisera without FloR1 protein were 1.151 and 0.0877, respectively.

family of the 12-TMS (transmembrane segment) MDR efflux pump systems (22).

Effects of the antibody on intracellular florfenicol accumulation and antimicrobial susceptibility. The JM109-R strain was originally constructed in order to eliminate the influence of other resistant genes (14). It showed almost the same resistance to florfenicol as the resistant wild-type clinical strains but had no resistance to other unrelated antibiotics tested (14). The intracellular accumulation of florfenicol of the clinical resistant isolate CVM1841 and the purposely constructed strain JM109-R with or without preincubation with the antibody is shown in Fig. 3. As expected, incubation of florfenicol-resistant isolates expressing the *floR* gene with florfenicol showed significantly lower intracellular florfenicol concentrations than those in florfenicol-sensitive strains. At steady state, the resistant strains CVM1841 and JM109-R accumulated about 3.5-fold less florfenicol than the sensitive strain JM109. Addition of the antisera to the bacterial culture resulted in a significant increase in intracellular florfenicol concentrations in the strains expressing the *floR* gene by approximately 40% (Fig. 3A and B) but not in the *floR*-negative strain (Fig. 3C). The results further support our previous conclusion that the *floR* gene confers resistance through an active efflux process (14). Binding of the antibody with the FloR protein decreased the activity of the efflux system.

The decreased efflux activity of the FloR protein could increase bacterial susceptibility to florfenicol. The MICs of florfenicol were determined for all *E. coli* strains to complement the florfenicol accumulation data. Antibody treatment of resistant strains decreased the MIC of florfenicol by two- to fourfold (Table 1). In contrast, incubation of the florfenicol-sensitive strains with the antibody had no effect on the MIC. The increased sensitivity of the resistant isolates to florfenicol after antibody treatment is consistent with the intracellular florfenicol accumulation results described above.

Detection of FloR protein by ELISA. The optimal amount of coating antigen (GST-FloR1) and antiserum dilution for the ELISA were determined as 300 ng/ml and 1:6,400, respectively. As shown in Fig. 4, the optical absorbance of approximately 1

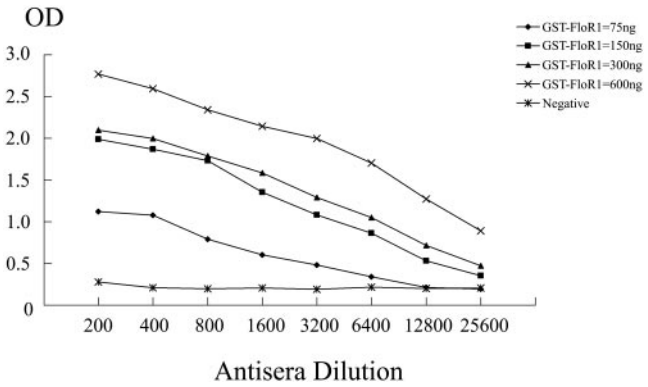

FIG. 4. ELISA absorbance values (at 450 nm) with various amounts of coating antigen (GST-FloR1) and a series of dilutions of the antisera. Serum from an unimmunized mouse was used as the negative control.

was obtained with 300 ng/ml coating antigen and 1:6,400-diluted antisera. The corresponding absorbance of the negative control was 0.216, giving a positive-control/negative-control ratio of 4.856, which was greater than 2.0. Therefore, 300 ng/ml coating antigen and 1:6,400-diluted antisera were selected as the optimal concentrations and were used in the ELISA for the determination of bacterial FloR concentrations.

The standard curve for FloR1 at 6.25 ng/ml to 200 ng/ml was linear (R^2 = 0.9979) (Fig. 5). FloR concentrations and corresponding optical density (OD) readings for florfenicol-sensitive and -resistant bacteria measured by the indirect competitive ELISA are shown in Table 1. Linear regression analyses using Microsoft Excel 2003 (Microsoft Corp.) showed a strong correlation between FloR protein concentrations and florfenicol MICs (R^2 = 0.9856; both parameters are log transformed) and between OD readings and MICs (R^2 = 9877; MICs are log transformed). The mean FloR protein concentration and OD readings of antisera without FloR1 protein were 1.03 (±0.67) ng/ml and 1.151 (±0.0877), respectively, compared to ≥19.2 (protein concentration) and ≤0.641 (OD) for florfenicol-resis-

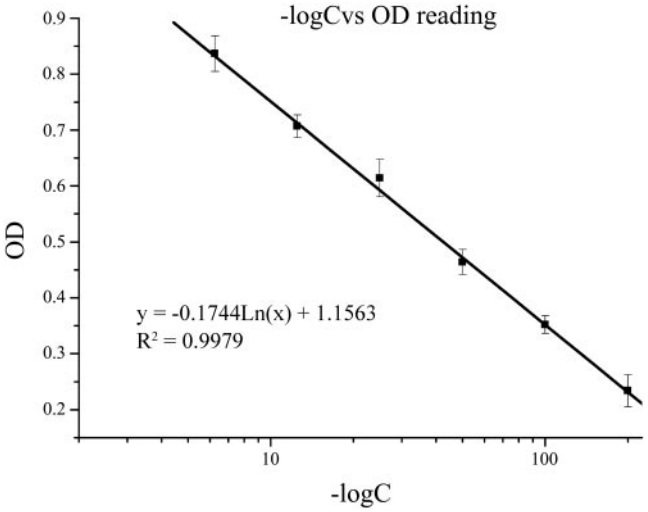

FIG. 5. Standard curve for FloR1 protein by ELISA.

tant isolates. The mean OD value minus three times the SD (i.e., an OD of 0.8978) or the mean FloR protein concentration plus three times the SD (i.e., 3.04 ng/ml) for *floR*-negative isolates may be considered the cutoff point for determining whether a bacterial isolate harbors the *floR* gene. However, because of the relatively small number of isolates analyzed in this study, a more reliable sensitivity/resistance cutoff value would require analysis of a larger number of *E. coli* isolates. The indirect competitive ELISA can be developed for use as a rapid assay tool to detect resistant bacteria.

The binding and accumulation assays showed that the antibody might act with the efflux pump protein and results in an increase in the accumulation of florfenicol in *E. coli* and an increase in bacterial susceptibility to florfenicol. The observation that the antibody may block drug efflux could be exploited as an intervention strategy against resistant strains. Using this anti-FloR1 antibody, we have developed a relatively rapid indirect ELISA method to detect clinical florfenicol-resistant bacterial isolates that harbor the *floR* gene, although further validation studies of field isolates using this method are required before it could be widely used. The ELISA is faster than the MIC assay and would provide results within 2 to 3 h.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Natural Science Foundation of China (30325032).

We thank David G. White from the FDA for donating the clinical strain CVM1841 and express gratitude to Jin Zhu, Therapeutic Goods Administration, Australia, for his revision of the manuscript.

REFERENCES

1. **Anthony, M. G., and R. M. Hall.** 2002. Efflux of chloramphenicol by the CmlA1 protein. FEMS Microbiol. Lett. **209:**209–213.
2. **Arcangioli, M. A., S. Leroy-Setrin, J. L. Martel, and E. Chaslus-Dancla.** 1999. A new chloramphenicol and florfenicol resistance gene flanked by two integron structures in *Salmonella typhimurium* DT104. FEMS Microbiol. Lett. **174:**327–332.
3. **Arcangioli, M. A., S. Leroy-Setrin, J. L. Martel, and E. Chaslus-Dancla.** 2000. Evolution of chloramphenicol resistance, with emergence of cross-resistance to florfenicol, in bovine *Salmonella* Typhimurium strains implicates definitive phage type (DT) 104. J. Med. Microbiol. **49:**103–110.
4. **Benoît, D., S. Schwarz, E. Nussbeck, S. Baucheron, J. Martel, E. Chaslus-Dancla, and A. Clochaert.** 2002. Molecular analysis of chromosomally florfenicol-resistant *Escherichia coli* isolates from France and Germany. J. Antimicrob. Chemother. **49:**49–54.
5. **Beoke, J. D., and P. Model.** 1982. A prokaryotic membrane anchor sequence: carboxyl terminus of bacteriophage f1 gene III protein retains it in the membrane. Proc. Natl. Acad. Sci. USA **79:**5200–5204.
6. **Bischoff, K. M., D. G. White, P. F. McDermott, S. Zhao, S. Gaines, J. J. Maurer, and D. J. Nisbet.** 2002. Characterization of chloramphenicol resistance in beta-hemolytic *Escherichia coli* associated with diarrhea in neonatal swine. J. Clin. Microbiol. **40:**389–394.
7. **Blickwede, M., and S. Schwarz.** 2004. Molecular analysis of florfenicol-resistant *Escherichia coli* isolates from pigs. J. Antimicrob. Chemother. **53:**58–64.
8. **Bolton, L. F., L. C. Kelley, M. D. Lee, P. J. Fedorka-Cray, and J. J. Maurer.** 1999. Detection of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J. Clin. Microbiol. **37:**1348–1351.
9. **Boyd, D., A. Cloeckaert, E. Chaslus-Dancla, and M. R. Mulvey.** 2002. Characterization of variant *Salmonella* genomic island 1 multidrug resistance regions from serovars Typhimurium DT104 and Agona. Antimicrob. Agents Chemother. **46:**1714–1722.
10. **Briggs, C. E., and P. M. Fratamico.** 1999. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob. Agents Chemother. **43:**846–849.
11. **Clochaert, A., S. Baucheron, and E. Chaslus-Dancla.** 2001. Nonenzymatic chloramphenicol resistance mediated by IncC plasmid R55 is encoded by a *floR* gene variant. Antimicrob. Agents Chemother. **45:**2381–2382.
12. **Clochaert, A., S. Baucheron, G. Flaujac, S. Schwarz, C. Kehrenberg, J. Martel, and E. Chaslus-Dancla.** 2000. Plasmid-mediated florfenicol resistance encoded by *floR* gene in *Escherichia coli* isolated from cattle. Antimicrob. Agents Chemother. **44:**2858–2860.
13. **Clochaert, A., K. S. Boumedine, G. Flaujac, H. Imberechts, I. D'Hooghe, and E. Chaslus-Dancla.** 2000. Occurrence of a *Salmonella enterica* serovar Typhimurium DT104-like antibiotic resistance gene cluster including the *floR* gene in *S. enterica* serovar Agona. Antimicrob. Agents Chemother. **44:**1359–1361.
14. **Du, X. D., C. Xia, J. Z. Shen, and B. B. Wu.** 2004. Characterization of florfenicol resistance among calf pathogenic *Escherichia coli*. FEMS Microbiol. Lett. **236:**183–189.
15. **Giraud, E., A. Cloeckaert, D. Kerboeuf, and E. Chaslus-Dancla.** 2000. Evidence for active efflux as primary mechanism of resistance to ciprofloxacin in *Salmonella enterica* serovar Typhimurium. Antimicrob. Agents Chemother. **44:**1223–1228.
16. **Hochhut, B., Y. Lotfi, D. Mazel, S. M. Faruque, R. Woodgate, and M. K. Waldor.** 2001. Molecular analysis of antibiotic resistance gene clusters in *Vibrio cholerae* O139 and O1 SXT constins. Antimicrob. Agents Chemother. **45:**2991–3000.
17. **Keyes, K., C. Hudson, J. J. Maurer, S. Thayer, D. G. White, and M. D. Lee.** 2000. Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. Antimicrob. Agents Chemother. **44:**421–424.
18. **Khan, A. A., M. S. Nawaz, S. A. Khan, and C. E. Cerniglia.** 2000. Detection of mutidrug-resistant *Salmonella typhimurium* DT104 by mutiplex polymerase chain reaction. FEMS Microbiol. Lett. **182:**355–360.
19. **Kim, E. H., and T. Aoki.** 1996. Sequence analysis of the florfenicol resistance gene encoded in the transferable R-plasmid of a fish pathogen, *Pasteurella piscicida*. Microbiol. Immunol. **40:**665–669.
20. **Lin, J., L. O. Michel, and Q. Zhang.** 2002. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. Antimicrob. Agents Chemother. **46:**2124–2131.
21. **National Committee for Clinical Laboratory Standards.** 1999. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
22. **Schwarz, S., C. Kehrenberg, B. Doublet, and A. Cloeckaert.** 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol. Rev. **28:**519–542.
23. **Shen, J., D. Hu, X. Wu, and J. R. Coats.** 2003. Bioavailability and pharmacokinetics of florfenicol in broiler chickens. J. Vet. Pharmacol. Ther. **26:**337–341.
24. **White, D. G., C. Hudson, J. J. Maurer, S. Ayers, S. Zhao, M. D. Lee, L. Bolton, T. Foley, and J. Sherwood.** 2000. Characterization of chloramphenicol and florfenicol resistance in *Escherichia coli* associated with bovine diarrhea. J. Clin. Microbiol. **38:**4593–4598.